Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice

Agathe Lambou Fotio1,*, Mireille Sylviane Dongmo Nguepi2, Roméo Joel Guemmogne Temdie3, Théophile Dimo4 and Télesphore Benoît Nguelfack5

1Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Buea, Cameroon
2Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, Buea, Cameroon
3Department of Biological Sciences, Faculty of Science, University of Ngaoundere, Ngaoundere, Cameroon
4Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde I, Yaoundé, Cameroon
5Department of Animal Biology, Faculty of Science, University of Dschang, Dschang, Cameroon

*Corresponding Author: Agathe Fotio Lambou, Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Buea, Cameroon.

Received: May 30, 2019; Published: October 31, 2019

Abstract

Liver diseases are very common worldwide. Intentional and non-intentional overdose of acetaminophen (APAP) are among the leading causes of acute liver failure. Bidens pilosa is a cosmopolitan, annual herb used in traditional medicine for its antioxidant, immuno-modulatory and hepatoprotective properties. The present study investigates the effect of Bidens pilosa aqueous extract on acetaminophen (APAP)-induced acute liver injury in mice.

Bidens pilosa leaves aqueous extract (100 and 200 mg/kg, p.o.), distilled water and ascorbic acid (50 mg/kg) were administered to mice 1 and 12 hours before acetaminophen (500 mg/kg, p.o.) treatment. Liver injury was evaluated by biochemical markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) serum activities, malondialdehyde (MDA), nitrite, and reduced glutathione (GSH) liver level, tumor necrosis factor alpha (TNF-α or TNF) and interleukin-1β (IL-1β) serum levels] and histological (H&E staining) analyses, 6 hours after acetaminophen administration.

Acetaminophen induced a gross appearance of liver injury with a significant (P < 0.01) increase of liver relative weight, ALT and AST serum activity, TNF and IL-1β serum content, nitrite and MDA liver level. In addition, GSH liver level was significantly depleted.

Prior administration of B. pilosa extract significantly decreased (P < 0.05) ALT and AST serum activity, TNF, IL-1β, MDA and nitrite level. GSH liver content was significantly (P < 0.05) increased by the plant extract. Histopathological examination of liver section indicated that B. pilosa extract remarkably reduced liver injury due to acetaminophen.

The results suggest that B. pilosa aqueous extract has hepatoprotective properties which could be mediated by anti-oxidant and anti-inflammatory activities.

Keywords: Acetaminophen; Liver Injury; GSH; Anti-Inflammatory; Anti-Oxidant

Abbreviations

B. pilosa: Bidens pilosa; APAP: Acetaminophen; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase; MDA: Malondialdehyde; NO: Nitric Oxide; GSH: Reduced Glutathione; TNF-α or TNF: Tumor Necrosis Factor Alpha; IL-1β: Interleukin-1 beta; IL-10: Interleukin-10; H&E: Hematoxylin/Eosin; NAC: N-Acetyl Cysteine; NAPQI: N-Acetyl-Para-Benzo-Quinone Imine

Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice

Introduction

Acute liver failure (ALF) also called fulminant hepatitis is a complex clinical syndrome that results from a sudden and severe loss in hepatocyte function due to massive hepatocyte necrosis in a patient without pre-existing liver disease [1-4].

World-wide viral hepatitis is the most frequent cause of ALF, followed increasingly by drugs and toxins [2,5,6]. Acetaminophen poisoning accounts for almost 50% of all cases of ALF in Western countries [3,7]. Current treatment options for acetaminophen poisoning are limited. N-acetyl cysteine is an effective antidote for acetaminophen overdose [7,8]. However, to effectively prevent the formation of toxic metabolite that leads to hepatic injury, N-acetyl cysteine should be administered as early treatment, following ingestion of higher doses of acetaminophen [7,9] providing 66% chance of recovery [9].

Therefore, novel therapeutic intervention strategies are needed. Nowadays, increasing efforts are directed towards medicinal plants for the development of new hepatoprotective drugs with minimum side effects. Several animal models are used to screen hepatoprotective effects of medicinal plants including carbon tetrachloride [10,11], d-(+)-galactosamine/lipopolysaccharide [12], acetaminophen alone [13] or the synergistic effect between acetaminophen and azithromycin-induced liver injury [14].

Bidens pilosa Linn is an erect, perennial herb belonging to Asteraceae’s family. The plant is widely distributed in temperate and tropical regions [15]. The hardiness, explosive reproductive potential and ability to thrive in almost any environment have enabled B. pilosa to establish throughout the world [16].

B. pilosa is either glabrous or hairy, with green opposite leaves. The plant is 60 cm to 150 cm height and prefers full sun and moderately dry soil. Flowers are white or yellow, and seeds long, narrow ribbed and black. B. pilosa propagates via seeds; a single plant producing 3000 - 6000 seeds [15].

B. pilosa is used in traditional medicine to treat diarrhea, dysentery, colic, infected wounds or burns and infections of respiratory system [16]. It is also used as antitumor, anti-inflammatory, anti-diabetic, anti-hyperglycemic, antioxidant, immune-modulatory, antimalarial, anti-bacterial, anti-fungal, anti-hypertensive, vasodilator, anti-ulcerative and hepatoprotective [17].

Phytochemical screenings of B. pilosa revealed the presence of polyacetylenes, flavonoids, essential oils, tannins, polysaccharides, phenols, amino acids, ascorbic acid, organic acids [16], phenylpropanoids, aromatics, porphyrins and terpenoids [15,17]. Phenolic compounds have been reported to exhibit strong antioxidant and hepatoprotective effects [18].

Previous studies shown that extracts from B. pilosa inhibited growth of many bacterial and fungal species in vitro [19], induced hypotension [20-22], possessed anti-hypertensive effect [23] and relaxed isolated vascular smooth muscle of rat [24]. Total flavonoids from B. pilosa protected mice and rats against carbon tetrachloride-induced liver injury [25]. In addition, many plants from Bidens genus have protective activities against carbon tetrachloride [26] and paracetamol-induced liver injury [27].

However, for the best of our knowledge, no scientific study has been carried out to elucidate the effect of B. pilosa extracts on acetaminophen-induced liver injury. The present work thus evaluates the effect of aqueous extract of Bidens pilosa leaves on acetaminophen-induced hepatotoxicity in mice.

Materials and Methods

Plant materials

Leaves of Bidens pilosa were harvested in Buea, South West Region, Cameroon, in November 2015, identified in the Limbe Botanical Garden, were a voucher specimen was deposited under the serial number SCA6352. The air dried leaves were reduced into powder. The powder (200g) was introduced into hot water (2L) and allowed to cool. The mixture was filtrated with Whatman paper N° 1 and water was evaporated in an oven at 40°C. The yield of B. pilosa water extract was 16.2%. Phytochemical screenings of B. pilosa have been previously reported [15,16]. They revealed the presence of polyacetylenes, flavonoids, essential oils, tannins, polysaccharides, phenols, amino acids, ascorbic acid, organic acids, phenylpropanoids, aromatics, porphyrins and terpenoids.

Chemicals and reagents

Citation: Agathe Lambou Fotio., et al.”Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice”. EC Pharmacology and Toxicology 7.11 (2019): 119-131.
Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice

Acetaminophen, trichloroacetic acid, thiobarbituric acid, sulphahilamide, naphthylethenediamide, phosphoric acid and DTNB were purchased from Sigma Aldrich (Germany). TNF-α, IL-1β and IL-10 kits were purchased from bio-technne (R&D Systems Europe Ltd). ALT, AST and ALP kits were purchased from Chronolab Systems, (Spain).

Animals
Male and female BALB/c mice (20 - 26g, 8 - 12 weeks old), obtained from the Animal House, Department of Zoology and Animal Physiology, Faculty of Science, University of Buea, Cameroon, were used for the investigations. They were housed six animals per cage under standard laboratory conditions (12:12h light/dark cycle at 25 ± 2°C), with free access to standard commercial diet and water. The experiment was carried out in accordance with institutional guidelines and approved by the Cameroon National Ethical Committee (Reg. N° FWAIRD 0001954).

Acetaminophen-induced liver injury in mice
Distilled water, Bidens pilosa aqueous extract (100 or 200 mg/kg, p.o.) or ascorbic acid (50 mg/kg, p.o.) were administered to mice (6 animals per group) 1 and 12h before induction of acute liver injury [12]. One hour after the second administration of test products, acetaminophen (500 mg/kg, p.o.) was administered to mice [28,29]. Ascorbic acid was administered at 50 mg/kg as previously reported [30]. Neutral control animals were treated only with distilled water. Blood samples were obtained from retro-orbital sinuses, 6 h after treatment with acetaminophen, and mice were sacrificed. Liver samples were collected for histological and biochemical analyses.

Histological analyses
Liver tissues obtained 6h after acetaminophen administration were fixed in 4% buffered formaldehyde and embedded in paraffin for hematoxylin/eosin staining (H&E).

Biochemical analyses
Biochemical analyses were done using serum and liver homogenate (20%) into Tris-HCl buffer (50 mM, pH 7.4).

Serum liver enzymes
Six hours after acetaminophen administration, hepatocyte damage was determined by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities in the serum with commercial kits (Chronolab), according to manufacturer’s instructions.

Reduced glutathione (GSH) amounts
Reduced glutathione (GSH) level in liver homogenate was assayed as described by Ellman [31]. Briefly, liver homogenate (20 µL) was added to 3 mL of Ellman reagent. The mixture was kept at room temperature for 1 hour, and the absorbance was read at 412 nm [32].

Malondialdehyde (MDA) level
MDA, an indicator of lipid peroxidation was determined in liver homogenate (20%). Briefly, liver homogenate supernatant (500 µL) was added to 250 µL of trichloroacetic acid solution (20%) and 500 µL of thiobarbituric acid solution (0.67%). The mixture was incubated at 90°C for one hour, cooled with tap water and centrifuged. Supernatant absorbance was measured at 530 nm. MDA was quantified by the extinction coefficient of 1.56 x 10^5 M/cm and expressed as µmol of MDA per g of tissue [32,33].

Nitric oxide (NO)/nitrite determination
Liver homogenate (20%) was used to measure nitrite level, as indicator of nitric oxide production, by Griess reagent (1% sulphamidine and 0.1% naphthylethenediamide in 2.5% phosphoric acid). Briefly, tissue homogenate (100 µL) was mixed with 100 µL of Griess reagent for 5 min. Absorbance was measured at 570 nm. Nitrite concentration was determined by comparison with a sodium nitrite standard curve [12,32].

TNF, IL-1β, IL-10 determination

**Bidens pilosa** Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice

TNF, IL-1β and IL-10 amounts in mice sera were assessed using enzyme-linked immunosorbent assay (ELISA) according to manufacturer’s instructions.

**Statistical analysis**

Values are expressed as mean ± SEM. Statistical differences between groups were determined using Analysis of Variance (ANOVA) followed by Dunnett’s test. *P* values less than 0.05 were considered significant.

**Results**

**Effect of Bidens pilosa extract on macroscopic aspect of acetaminophen treated mice liver**

Macroscopic examination of acetaminophen-treated mice liver showed organ swollen with significant (*P* < 0.01) increase of liver relative weight, compared to control mice, treated with distilled water (Figure 1). In addition, acetaminophen treatment resulted in massive hepatic toxicity as revealed by liver gross morphology (Figure 2). Prior administration of *B. pilosa* (100 or 200 mg/kg) or ascorbic acid significantly (*P* < 0.05) prevented acetaminophen to induce liver swollen, reducing massive hepatotoxicity, as morphologically observed.

**Histopathological examination of liver sections**

Liver section of neutral mice showed cells and their nuclei relatively uniform in size and staining characteristics, with normal appearance of hepatic sinusoids (Figure 3A). Histological micrograph of acetaminophen-treated mice showed infiltration of inflammatory cells in liver parenchyma, dilated sinusoids, loss of classical arrangement of hepatic cords, nuclei darkly stained, indicating pyknotic changes and clumping of nuclear material (Figure 3B). Administration of *B. pilosa’s* aqueous extract (100 or 200 mg/kg) or ascorbic acid resulted in reduction of liver injury with a noticeable improvement of histopathological parameters (Figure 3C-3E).

---

**Figure 1:** Effect of *B. pilosa* aqueous extract on liver relative weight after acetaminophen treatment.

###P < 0.01 compared to control animals treated with distilled water; *P < 0.05, **P < 0.01, compared to animals treated with distilled water and acetaminophen (500 mg/kg), Acetam: Acetaminophen, B. p: Bidens pilosa, AA: Ascorbic Acid.

---

*Citation:* Agathe Lambou Fotio., *et al.* "Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice". *EC Pharmacology and Toxicology* 7.11 (2019): 119-131.
Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice


**Figure 2:** Macroscopic views of acetaminophen-treated mice livers: Effect of *B. pilosa* extract.
A: Liver of mice treated with distilled water; B: Liver of mice treated with distilled water and acetaminophen (500 mg/kg), C: Liver of mice treated with *B. pilosa* (100 mg/kg) and acetaminophen (500 mg/kg), D: Liver of mice treated with *B. pilosa* (200 mg/kg) and acetaminophen (500 mg/kg); E: Liver of mice treated with ascorbic acid (50 mg/kg) and acetaminophen (500 mg/kg).

**Figure 3:** Reduction of histopathological changes in liver tissue of acetaminophen-treated mice by *B. pilosa* extract.
A: Liver section of mice treated with distilled water, cells are relatively uniform in size and staining characteristics. B: Histological micrograph of acetaminophen-treated mice showing infiltration of inflammatory cells in liver parenchyma, dilated sinusoids, nuclei darkly stained, indicating pyknotic changes. C, D & E: Histological micrographs of liver section of mice treated with *B. pilosa* (100 and 200 mg/kg) or ascorbic acid (50 mg/kg) respectively, followed by acetaminophen (500 mg/kg). Administration of *B. pilosa*’s extract or ascorbic acid resulted in reduction of liver injury (H&E 400×).
Effects of *B. pilosa* aqueous extract on liver enzymes

Six hours after acetaminophen administration to mice, there was eleven folds and six folds increase of ALT and AST serum activity, respectively, compared to neutral control (treated with distilled water). ALP serum activity was also significantly (P < 0.01) increased after acetaminophen administration. ALT, AST and ALP serum activities were significantly (P < 0.05, P < 0.01) reduced by *B. pilosa* extract and ascorbic acid (Figure 4).

**Figure 4:** Reduction of liver enzymes (A: ALT, B: AST, C: ALP) activity in acetaminophen treated mice by *B. pilosa* extract. Results are represented as means ± SEM, n=6. ##P < 0.01, ###P < 0.001, compared to control animals treated with distilled water; *P < 0.05, **P < 0.01, ***P < 0.001, compared to animals treated with distilled water and acetaminophen (500 mg/kg). ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Alkaline Phosphatase, Acetam: Acetaminophen, B. p: Bidens pilosa, AA: Ascorbic Acid.

*Citation:* Agathe Lambou Fotio, et al. "*Bidens pilosa* Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice". *EC Pharmacology and Toxicology* 7.11 (2019): 119-131.
Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice

Effect of *B. pilosa* aqueous extract on reduced glutathione (GSH) level

GSH liver level was significantly (P < 0.001) decreased by acetaminophen treatment, compared to neutral animals (treated with distilled water). Prior administration of *B. pilosa* extract (100 or 200 mg/kg) or ascorbic acid significantly (P < 0.05) increased GSH liver level, compared to acetaminophen-treated mice (Figure 5).

![Figure 5](image)

**Figure 5:** Effect of *B. pilosa* extract on GSH liver level in acetaminophen-treated mice. Results are represented as means ± SEM, n=6. ###P < 0.001 compared to control animals treated with distilled water; *P < 0.05, **P < 0.01, compared to animals treated with distilled water and acetaminophen (500 mg/kg). Acetam: Acetaminophen, B. p: Bidens pilosa, AA: Ascorbic Acid.

Reduction of malondialdehyde (MDA) level by *B. pilosa* aqueous extract

Administration of acetaminophen resulted in significant (P < 0.01) increase of lipid peroxidation, evaluated by MDA level in liver homogenate. Pre-treatment of mice with *B. pilosa* extract resulted in significant (P < 0.01) reduction of lipid peroxidation. Plant extract effect was comparable to that of ascorbic acid (Figure 6).

![Figure 6](image)

**Figure 6:** Inhibition of acetaminophen-induced liver lipid peroxidation by *B. pilosa* extract. Results are represented as means ± SEM, n=6. ##P < 0.01 compared to control animals treated with distilled water; **P < 0.01, compared to animals treated with distilled water and acetaminophen (500 mg/kg). Acetam: Acetaminophen, B. p: Bidens pilosa, AA: Ascorbic Acid.

Citation: Agathe Lambou Fotio, *et al.* "Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice". *EC Pharmacology and Toxicology* 7.11 (2019): 119-131.
Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice

Effect of *B. pilosa* aqueous extract on nitrite accumulation in liver of acetaminophen-treated mice

Six hours after administration of acetaminophen, nitrite level in the liver was significantly ($P < 0.01$) increased, compared to neutral animals (treated with distilled water). Aqueous extract of *B. pilosa* (100 or 200 mg/kg) or ascorbic acid significantly ($P < 0.01$) reduced nitrite liver’s level (Figure 7).

![Figure 7: Reduction of liver nitrite level in acetaminophen treated mice by *B. pilosa* extract. Results are represented as means ± SEM, n=6. ###$P < 0.001$ compared to control animals treated with distilled water; **$P < 0.01$, ***$P < 0.001$, compared to animals treated with distilled water and acetaminophen (500 mg/kg), Acetam: Acetaminophen, B. p: Bidens pilosa, AA: Ascorbic Acid.](image)

TNF, IL-1β, IL-10 determination

Administration of acetaminophen to mice induced systemic release of pro-inflammatory cytokines (TNF and IL-1β) as well as IL-10, an anti-inflammatory cytokine. As shown in figure 8, TNF and IL-1β serum levels were significantly reduced by *B. pilosa* extract. IL-10 was significantly reduced only by the lower dose (100 mg/kg) of plant extract.

Discussion

The present study was aimed to evaluate *Bidens pilosa*’s leaves extract on acetaminophen-induced hepatotoxicity in mice. *B. pilosa*’s extract reduced ALT and AST serum activity, TNF, IL-1β serum level, MDA and nitrite levels and increased GSH concentration in acetaminophen-treated mice liver.

Liver plays principal role in detoxification of various drugs and xenobiotic. During detoxification pathway, cytochrome P-450-2E1 reacts with acetaminophen, depletes glutathione, and generates reactive oxygen and nitrogen species and N-acetyl-p-benzoquinone imine (NAPQI). This results in direct hepatotoxicity [6-8]. Oxidative stress due to imbalance between antioxidant and oxidant molecules affects lipids in cells [5], inducing lipid peroxidation [34]. Administration of *B. pilosa*’s extract inhibited acetaminophen-induced lipid peroxidation and increased GSH level in mice liver. Results suggest that *B. pilosa*’s extract either reduces formation of NAPQI from acetaminophen metabolism, or stimulates GSH repletion, providing surplus cysteine for Krebs cycle and contributing for scavenging of free radicals and peroxynitrite [7,35]. Present results corroborate those previously obtained by Singh., et al. [14], who showed that protection against liver injury by a plant extract is related to its anti-oxidant properties with reduction of lipid peroxidation, and increase of GSH level.

Citation: Agathe Lambou Fotio., et al."Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice". *EC Pharmacology and Toxicology* 7.11 (2019): 119-131.
Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice

**Figure 8:** Effect of B. pilosa extract on TNF, IL-1β and IL-10 serum level in acetaminophen-treated mice.

Results are represented as means ± SEM, n=6. #P < 0.05, ##P < 0.01, compared to control animals treated with distilled water; *P < 0.05, compared to animals treated with distilled water and acetaminophen (500 mg/kg). Acetam: Acetaminophen, B. p: Bidens pilosa, AA: Ascorbic Acid.

_Citation:_ Agathe Lambou Fotio, _et al._ "Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice_. _EC Pharmacology and Toxicology_ 7.11 (2019): 119-131.
Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice

Acetaminophen overdose is also associated to increase NO synthesis in liver tissue. *B. pilosa* extract reduced nitrite concentration in liver tissue. Inhibition of NO production can be a useful therapeutic strategy against hepatitis [36].

Hepatocytes swelling results in increase of liver’s weight due to acetaminophen. Vacuolization, inflammation and release of alanine aminotransferase are other key processes of acetaminophen-induced hepatocytes necrosis [7]. Prior administration of *B. pilosa*’s extract inhibited liver swelling and reduced ALT and AST serum activity. Inhibition of liver enzymes activity correlated with reduction of neutrophils accumulation in liver parenchyma, as well as reduction of pyknosis and clumping of nuclear material due to acetaminophen. Neutrophils accumulation in liver parenchyma [37], pyknosis and clumping of nuclear material have been associated to acetaminophen-induced liver injury [38].

Inflammatory mediators like TNF and NO are involved in animal models of liver injury, including acetaminophen [5] and o-galactosamine/lipopolysaccharide-induced liver injury [12]. Inhibition of iNOS expression and TNF production correlated with decrease hepatotoxicity in both models of liver injury. IL-1β signaling has also been implicated in potentiation of acetaminophen-induced hepatotoxicity [39]. IL-10 is an anti-inflammatory cytokine that is known to modulate pro-inflammatory response in hepatic injury [5,40].

Results corroborate those previously obtained [25,41], showing that total flavonoids content of *B. pilosa* and *B. bipinnata*, respectively, protect mice and rats against CCl₄-induced liver injury, reducing ALT, AST serum activity and MDA level, superoxide dismutase and glutathione peroxidase activities in the liver. Authors concluded that both plants effects are related, at least in part, to their antioxidant properties. Presence of flavonoids and other phenolic compounds has been revealed in *B. pilosa* [16]. Effects of *B. pilosa* extract may be attributed to phenolic compounds which have been reported to exhibit strong antioxidant and hepatoprotective effects [18].

Conclusion
From these investigations we demonstrate that *Bidens pilosa* aqueous extract inhibits acetaminophen-induced hepatotoxicity. These effects appear to be mediated by GSH repletion, inhibition of TNF, IL-1β, nitric oxide synthesis, lipid peroxidation and accumulation of inflammatory cells in liver parenchyma. Considering these results, aqueous extract of *B. pilosa* has hepatoprotective activity due to its anti-oxidant and anti-inflammatory properties. Results strongly support ethno-pharmacological uses of *B. pilosa* against liver diseases. However, further investigations are required to purify and identify active compound (s) in this extract.

Declarations of Interest
The authors declare that there is no conflict of interest regarding the publication of this article.

Authors’ Contributions
ALF, TBN, RJGT and TD designed the study. ALF and MSDN performed the experiments. ALF was a major contributor in writing the manuscript. TBN, RJGT, MSDN, and TD critically revised the manuscript. All authors read and approved the manuscript for submission.

Funding
This work was supported by Grants (F/5548-1, 2014) to AFL, from International Foundation for Science (IFS).

Acknowledgments
The authors are very thankful to the International Foundation for Science (IFS). The authors are very thankful to the University of Buea (Cameroon).

Bibliography

Citation: Agathe Lambou Fotio., et al. "Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice". *EC Pharmacology and Toxicology* 7.11 (2019): 119-131.
Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice


Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice


Bidens pilosa Extract Effectively Alleviates Acetaminophen-Induced Hepatotoxicity in Mice


Volume 7 Issue 11 November 2019
©All rights reserved by Agathe Lambou Fotio., et al.