

Protective Effects of Fraction F3 from *Desmodium velutinum* Leaves on Histopathological Alterations in Rat Vital Organs

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Abstract:

Medicinal plants constitute an important source of bioactive compounds with antioxidant and organ-protective properties and continue to play a central role in traditional medicine. This study investigated the protective effects of fraction F3 isolated from the hydroethanolic leaf extract of *Desmodium velutinum* against experimentally induced cardiac, hepatic, and renal injuries in rat. Fraction F3 was selected for the present study because it exhibited the highest pharmacological activity among all the fractions obtained during the bio-guided fractionation of the hydroethanolic extract of *Desmodium velutinum* leaves. Based on these preliminary findings, F3 was considered the most promising fraction for further investigation. The hydroethanolic extract of *D. velutinum* leaves underwent bio-guided fractionation. The daily administration of paracetamol (500 mg/kg, per os), gentamicin (80 mg/kg, i.p.), and ethanol (30%, per os): induced marked histopathological alterations, including cardiac congestion, periportal hepatic necrosis, renal tubular atrophy, vascular congestion, and inflammatory cell infiltration. Ethanol-induced lesions were the most severe, followed by paracetamol- and gentamicin-induced injuries. However, rats treated with paracetamol (500 mg/kg) combined with fraction F3 (25 and 50 mg/kg), gentamicin (80 mg/kg) combined with fraction F3 (25 and 50 mg/kg), and ethanol (30 %) combined with fraction F3 (25 and 50 mg/kg) showed a total improvement in vital organs, indicating a reversible effect. This fraction appears to protect vital organs from the toxicity induced by paracetamol, gentamicin, and ethanol. Fraction F3 was characterized by high levels of polyphenols and flavonoids and exhibited strong DPPH radical-scavenging activity.

Keywords: Protective effects, vital organs, *Desmodium velutinum*, fraction, rats.

Effets protecteurs de la fraction F3 issue des feuilles de *Desmodium velutinum* sur les altérations histopathologiques des organes vitaux chez le rat

Résumé :

Les plantes médicinales constituent une source importante de composés bioactifs dotés de propriétés antioxydantes et organoprotectrices, et continuent de jouer un rôle central dans la médecine traditionnelle. Cette étude a examiné les effets protecteurs de la fraction F3, isolée à partir de l'extrait hydroéthanolique des feuilles de *Desmodium velutinum*, contre les lésions cardiaques, hépatiques et rénales induites expérimentalement chez le rat. La fraction F3 a été sélectionnée dans la présente étude car elle présentait l'activité pharmacologique la plus élevée parmi toutes les fractions obtenues lors du fractionnement guidé par l'activité biologique de l'extrait hydroéthanolique des feuilles de *Desmodium velutinum*. Sur la base de ces résultats préliminaires, la fraction F3 a été considérée comme la plus prometteuse pour des investigations ultérieures. L'extrait hydroéthanolique des feuilles de *D. velutinum* a fait l'objet d'un fractionnement guidé par l'activité biologique. L'administration quotidienne de paracétamol (500 mg/kg, par voie orale), de gentamicine (80 mg/kg, par voie intrapéritonéale) et d'éthanol (30 %, par voie orale) a provoqué des altérations histopathologiques marquées, notamment une congestion cardiaque, une nécrose hépatique périportale, une atrophie tubulaire rénale, une congestion vasculaire et une infiltration de cellules inflammatoires. Les lésions induites par l'éthanol étaient les plus sévères, suivies par celles causées par le paracétamol et la gentamicine. Toutefois, les rats traités par le paracétamol (500 mg/kg), la gentamicine (80 mg/kg) ou l'éthanol (30 %) en association avec la fraction F3 (25 et 50 mg/kg) ont présenté une amélioration totale de l'état des organes vitaux, témoignant d'un effet réversible. Cette fraction semble protéger les organes vitaux contre la toxicité induite par le paracétamol, la gentamicine et l'éthanol. La fraction F3 se caractérisait par une teneur élevée en polyphénols et en flavonoïdes et a manifesté une forte activité de piégeage des radicaux DPPH.

Mots-clés : Effets protecteurs, organes vitaux, *Desmodium velutinum*, fraction, rats.

Introduction

Vitellaria In the context of contemporary health, medicinal plants play a crucial role in the advancement of traditional medicine, mainly thanks to their rich array of properties and their intrinsic ability to generate numerous bioactive substances. In Africa, traditional medicine is a

fundamental pillar of medical practice in rural areas.

WHO (2002) estimates that 80% of the African population rely on traditional medicine for their health, with most therapies based on the active principles of medicinal plants. These plant

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species, of great importance to the health of populations, deserve to be studied scientifically in order to optimize their use (Biyiti *et al.*, 2004).

The use of plant extracts containing bioactive constituents has become an essential approach in preventive medicine (Keita *et al.*, 2004). Their medicinal properties depend on the presence of various bioactive agents belonging to different chemical classes.

They are considered a veritable reservoir of irreplaceable molecules (Kumaraswamy *et al.*, 2008). In several studies, these molecules have demonstrated essential biological activities for the body, including antioxidant (Kong *et al.*, 2010), hepatoprotective, cardioprotective and nephroprotective activity (Bruneton, 2003; El Akrimi Safia, 2015).

Over the past 20 years, more than 25% of drugs have been isolated directly from plants, while the remaining 75% come from their chemical derivatives. In vitro data and clinical trials show that medicinal plants and their active ingredients are increasingly used as alternative therapies, particularly in patients with chronic diseases such as kidney disease (Lai *et al.*, 2010).

1. Materials and Methods

1.1. Materials

1.1.1. Plant Material

In the present study, *Desmodium velutinum* leaves were used. They were harvested in November 2018 in the village of Sossi, in the Niari department (Congo). The samples were identified at the Institut national de Recherche en Sciences Exactes et Naturelles (IRSEN), in the national herbarium department, under number 636. These samples were then dried for 12 days sheltered from the sun, in the laboratory of the National Institute of Health Science Research (IRSSA).

1.1.2. Animal material

The animal models consisted of male and female Wistar rats weighing between 250 and 270 g. The animals were housed at the animal facility of the National Institute for Research in Health Sciences (IRSSA) under controlled conditions (ambient temperature of 27 ± 1 °C, 12-hour light/dark cycle) with ad libitum access to standard rat chow and tap water.

1.2. Methods

1.2.1. Fraction Preparation

Following this elution, the F3 fraction was obtained, which is the subject of this study. The hydroethanol extract of *D. velutinum* leaves was subjected to bioguided fractionation. Hydroethanolic extract: 50% ethanol and 50%

However, the use of certain drugs, such as paracetamol, antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), psychotropic drugs and lipid-lowering drugs, can lead to cardiac, hepatic and renal toxicity (Sgro *et al.*, 2002).

Taking into account the side effects and damage caused by certain so-called synthetic drugs, the application of medicinal plant extracts is a promising avenue in the fight against certain diseases (Deteix, 2005).

Hence the importance of directing research towards new avenues, particularly medicinal plants, which have always been a source of inspiration for new medicines.

Despite the toxicity caused by certain exogenous agents, some medicinal plants are rich in polyphenolic compounds that confer strong anti-radical activity against DPPH, thereby ensuring the protection of vital organs.

This is the background to the present study, which aims to evaluate the protective effects of fraction F3 from the aqueous extract of *Desmodium velutinum* (Willd.) D.C. (Fabaceae) leaves on vital organs in rats.

water (v/v), and F3: 70 % ethanol and 30% water (v/v). To this end, 1 g of the extract was chromatographed on an open polyamide 6 column (100 g) (Fluka), 15 mm in diameter and 50 cm long.

Elution was carried out with a mixture of water (F1), water-ethanol (F2 and F3) and 100% ethanol (F4) of decreasing polarity, in the following proportions: 100% H₂O, EtOH-H₂O (30 :70 v/v), EtOH-H₂O (70 :30 v/v), and 100 % EtOH. Follow-up elution yielded fraction F3, the subject of this study.

1.2.2. Evaluation of the Protective Effects of Fraction F3 in Rats

1.2.2.1. On Paracetamol-Induced Toxicity

Five (5) lots of 6 rats were selected for the experiment, divided as follows:

- lot 1 received distilled water at 1 mL/100 g ;
- lot 2 received paracetamol at 500 mg/kg (per os) for 14 days ;
- lot 3 received paracetamol at 500 mg/kg + fraction F3 (25 mg/kg, per os) for 14 days ;
- lot 4 received paracetamol at 500 mg/kg + fraction F3 (50 mg/kg, per os) for 14 days ;
- lot 5 received paracetamol at 500 mg/kg + Legalon (70 mg/kg, per os) for 14 days.

1.2.2.2. On Gentamicin-Induced Toxicity

Four (4) lots of 6 rats were selected for the experiment, divided as follows:

- lot 1 received distilled water at 1 mL/100 g (per os) for 7 days ;
- lot 2 received distilled water at 1 mL/100 g for the first 3 days, followed by gentamicin solution (80 mg/kg, i.p.) starting on day 4, administered for 7 days ;
- lot 3 received gentamicin (80 mg/kg, i.p.) + fraction F3 (25 mg/kg, per os) for 7 days ;
- lot 4 received gentamicin (80 mg/kg, i.p.) + fraction F3 (50 mg/kg, per os) for 7 days.

1.2.2.3. On Ethanol-Induced Toxicity

Five (5) lots of 6 rats were selected for the experiment, divided as follows :

- lot 1 received distilled water at 1 mL/100 g (per os) for 8 weeks ;
- lot 2 received ethanol 30% (2 mL/kg/day, per os) + distilled water (1 mL/100 g/day, per os) for 8 weeks ;
- lot 3 received ethanol 30% (2 mL/kg/day, per os) + fraction F3 (25 mg/kg, per os) for 8 weeks ;
- lot 4 received ethanol 30% (2 mL/kg/day, per os) + fraction F3 (50 mg/kg, per os) for 8 weeks ;
- lot 5 received ethanol 30% (2 mL/kg/day, per os) + Legalon (70 mg/kg).

1.2.3. Anatomical and histopathological parameters

2. Resultats

2.1. Evaluation of the protective effects of fraction F3 in paracetamol-treated rats

2.1.1. Anatomical-histopathological study of the heart

Histological examination of the hearts of rats treated with paracetamol (500 mg/kg) for 14 days revealed the presence of an abnormal myocardial bundle, with atrophy of the myocardial cells (Fig. 1 D). Rats treated with paracetamol (500 mg/kg) and the F3 fraction of *D. velutinum* leaves at doses of 25 and 50 mg/kg showed a general improvement in heart architecture (fig. 1 G and J), close to that of control rats (fig. 1V).

2.1.2. Anatomical-histopathological study of the liver

Figure 2 shows the effects of paracetamol (500 mg/kg) on histopathological sections of the livers of rats treated for 14 days.

Overall, the livers of paracetamol-treated rats showed more inflammatory cells and periportal necrosis (Fig. 2 E) than those of control rats (Fig. 2 B).

Histological sections were prepared in several stages, using the classic method of anatomo-histopathological study (Fléjou and Cervera, 2009; Caruana, *et al.*, 2016).

1.2.4. Thin layer chromatography (TLC)

Phenolic compounds were characterized by thin-layer chromatography (TLC). A small quantity (0.5g) of F3 was deposited on the stationary phase, which then comes into contact with the mobile phase (eluent). The mobile phase migrated upwards by capillary action along the fixed phase, entraining the constituents of the mixture, resulting in separation of the constituents of the mixture to be analyzed. Each component migrated to a specific height, characterized by a frontal ratio (Lagnika, 2005).

1.2.5. Anti-radical activity on Thin-Layer Chromatography

This chromatographic technique was carried out on the fraction F3 of *D. velutinum* leaves. Elution was carried out using a system composed of ethyl acetate, formic acid and water (8:1:1).

The chromatographic plate was then sprayed with DPPH (2,2- Diphenyl-1-Picryl-Hydrazyl), dried and observed in the open air (Hennebelle Thierry, 2006).

In rats treated with paracetamol (500 mg/kg) and the F3 fraction of *D. velutinum* leaves (25 and 50 mg/kg), there was a clear appearance of the hepatic artery branch and the hepatic vein branch (fig. 2 H and K).

2.1.3. Anatomo-histopathological study of the kidneys

Treatment with paracetamol (500 mg/kg) for 14 days induced a cluster of inflammatory cells in the kidneys of rats (Fig. 3 F). In rats treated with paracetamol plus the F3 fraction of *D. velutinum* leaves (25 and 50 mg/kg), their kidneys (Figure 3 I and L) showed a morphology very similar to that of control rats (Figure 3 C).

2.1.4. Anatomical-histopathological study of the kidneys

Treatment with paracetamol (500 mg/kg) for 14 days induced a cluster of inflammatory cells in the kidneys of rats (Fig. 3 F). In rats treated with paracetamol plus the F3 fraction of *D. velutinum* leaves (25 and 50 mg/kg), their kidneys (Figure 3 I and L) showed a morphology very similar to that of control rats (Figure 3 C).

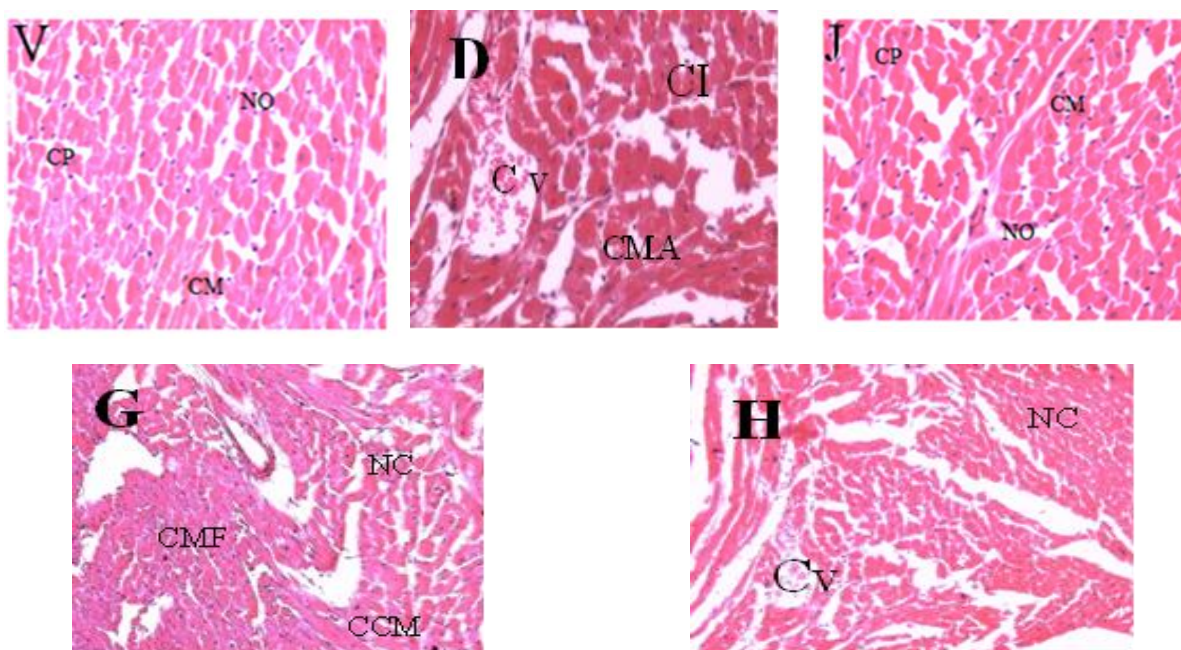


Figure 1: Histological section of the heart

F3: Fraction from *D. velutinum* leaves ; **V:** Control heart ; **D:** Heart of rats treated with paracetamol (500 mg/kg) ; **G:** Heart of rats treated with paracetamol + F3 (25 mg/kg) ; **J:** Heart of rats treated with paracetamol + F3 (25 mg/kg) ; **H:** Heart of rats treated with paracetamol + Legalon (70 mg/kg) ; **No:** Nucleus ; **CM:** Myocardial cells ; **CP:** Cytoplasm ; **CCM:** Cytoplasm of myocardial cells ; **CMN:** Normal myocardial cells ; **CMA:** Atrophied myocardial cells ; **NC:** Nucleus of the cells ; **CMF:** Myocardial bundle ; **Staining:** Hematoxylin-Eosin x40.

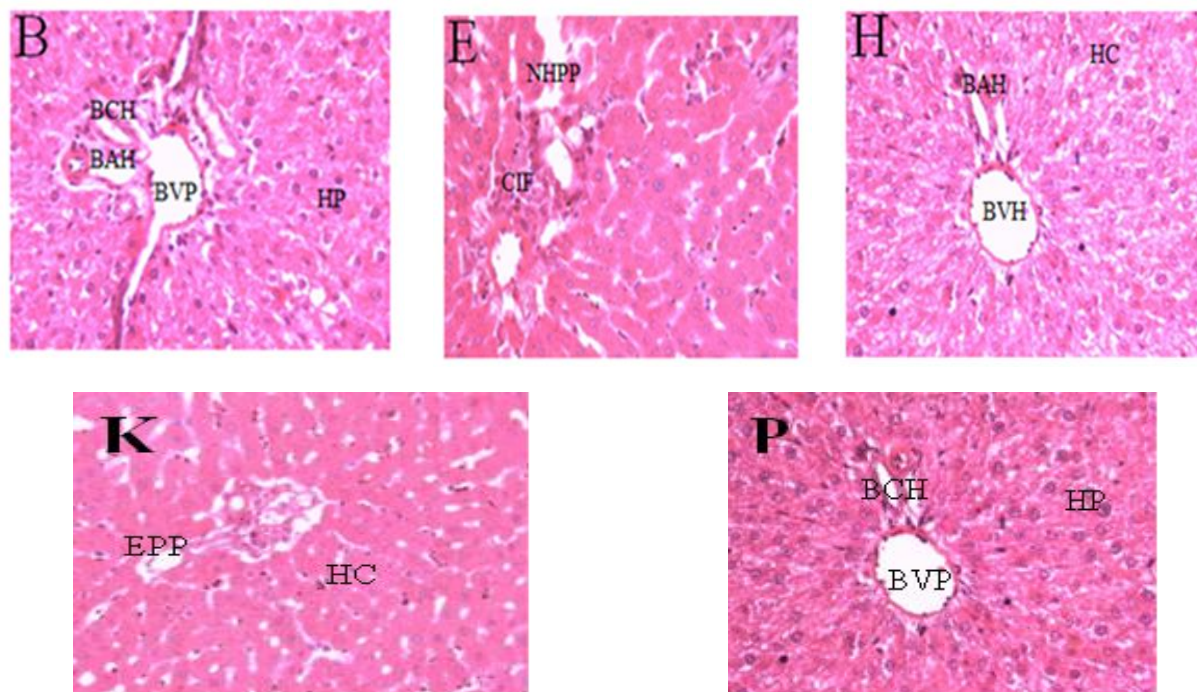


Figure 2: Histological section of the liver

F3: Fraction from *D. velutinum* leaves ; **B:** Control liver ; **E:** Liver of rats treated with paracetamol (500 mg/kg) ; **H:** Liver of rats treated with paracetamol (500 mg/kg) + fraction F3 (25 mg/kg) ; **K:** Liver of rats treated with paracetamol (500 mg/kg) + fraction F3 (50 mg/kg) ; **P:** Liver of rats treated with paracetamol + Legalon ; **BCH:** Hepatic duct branch ; **BAH:** Hepatic artery branch ; **BVH:** Hepatic vein branch ; **HP:** Hepatocytes ; **CIF:** Inflammatory cells ; **FPP:** Periportal fibrosis ; **HC:** Hepatocytes ; **Staining:** Hematoxylin-Eosin x40.

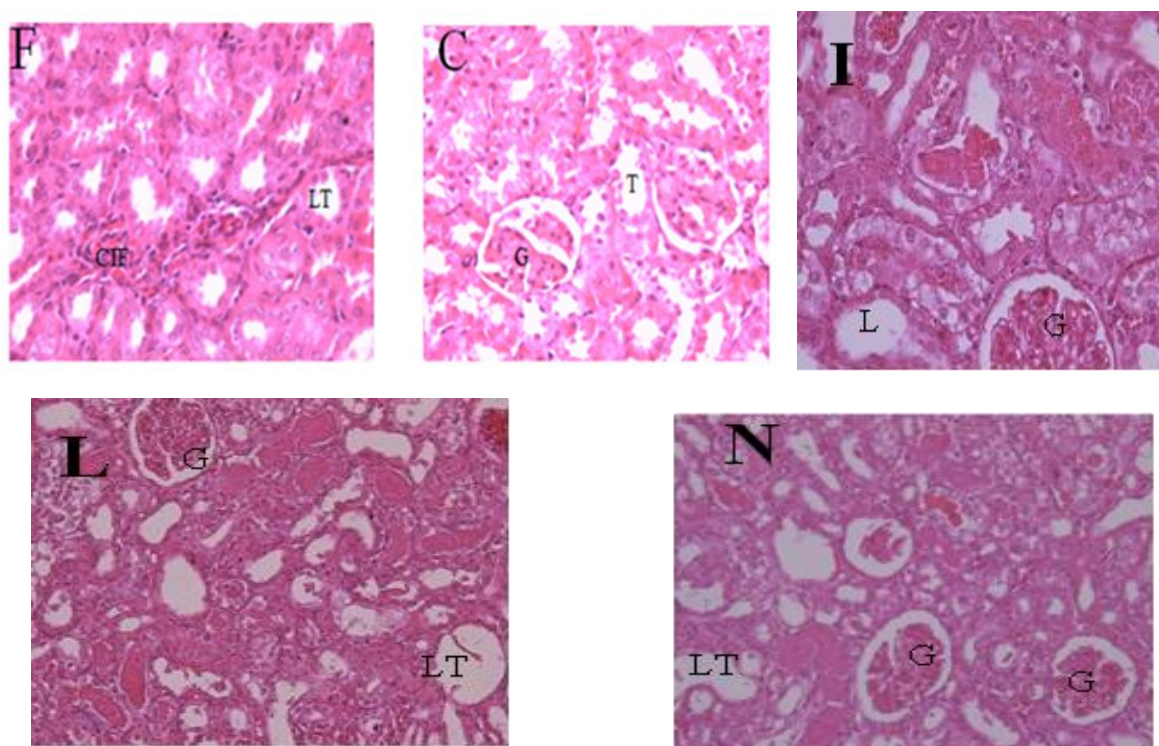


Figure 3: Histological section of the Kidney

F3: Fraction from *D. velutinum* leaves; **C:** Control kidney; **F:** Kidney of rats treated with paracetamol (500 mg/kg) ; **I:** Kidney of rats treated with paracetamol + F3 (25 mg/kg); **L:** Kidney of rats treated with paracetamol + F3 (50 mg/kg); **N:** Kidney of rats treated with paracetamol + Legalon (70 mg/kg); **G:** Glomerulus; **T:** Tubule; **LT:** Tubule lumen; **CIF:** Inflammatory cells; **Staining:** Hematoxylin-Eosin x40.

2.2. Evaluation of the protective effects of fraction F3 in rats treated with gentamicin

2.2.1. Anatomopathological study of the heart

In rats treated with gentamicin (80 mg/kg) and distilled water for 10 days, cardiac congestion was observed (Figure 4Y), along with the presence of multiple inflammatory cells throughout the myocardium.

However, in rats treated with fraction F3 from *D. velutinum* leaves (50 mg/kg) (Figure 4 B* and K*), the heart structure closely resembled that of the control rats (Figure 4V).

2.2.2. Anatomopathological study of the liver

Treatment with gentamicin (80 mg/kg) for ten days in rats led to the infiltration of inflammatory cells in the liver, accompanied by atrophy of the portal space and the hepatic artery branch (Figure 5G*).

In contrast, rats treated simultaneously with gentamicin (80 mg/kg) and fraction F3 from *D. velutinum* leaves at the dose of 50 mg/kg exhibited a normal renal architecture, resembling that of the control rats (Figure E*).

However, rats that received fraction F3 at the dose of 50 mg/kg showed periportal fibrosis in

the kidneys, along with multiple inflammatory foci (Figure 5Z).

2.2.3. Anatomopathological study of the kidneys

The administration of gentamicin (80 mg/kg) for 10 days in rats led to intratubular hemorrhages throughout the glomerulus, along with tubular dystrophy (Figure 6M*).

In rats treated with gentamicin + fraction F3 from *D. velutinum* leaves, there was a reduction in tubular spaces, followed by a significant restoration of glomerular morphology, resembling that of control rats (Figures 6D and 6W**).

2.3. Evaluation of the protective effects of fraction F3 in rats treated with ethanol

2.3.1. Anatomopathological study of the heart

Figure 7 illustrates the effects of 30% ethanol on the anatomical and histological parameters of rats treated with ethanol for 8 weeks. This figure shows that rats treated with 30% ethanol and distilled water exhibit vascular congestion throughout the myocardium, along with the presence of multiple inflammatory cells (Figure 7M), in contrast to the control rats (Figure 7A).

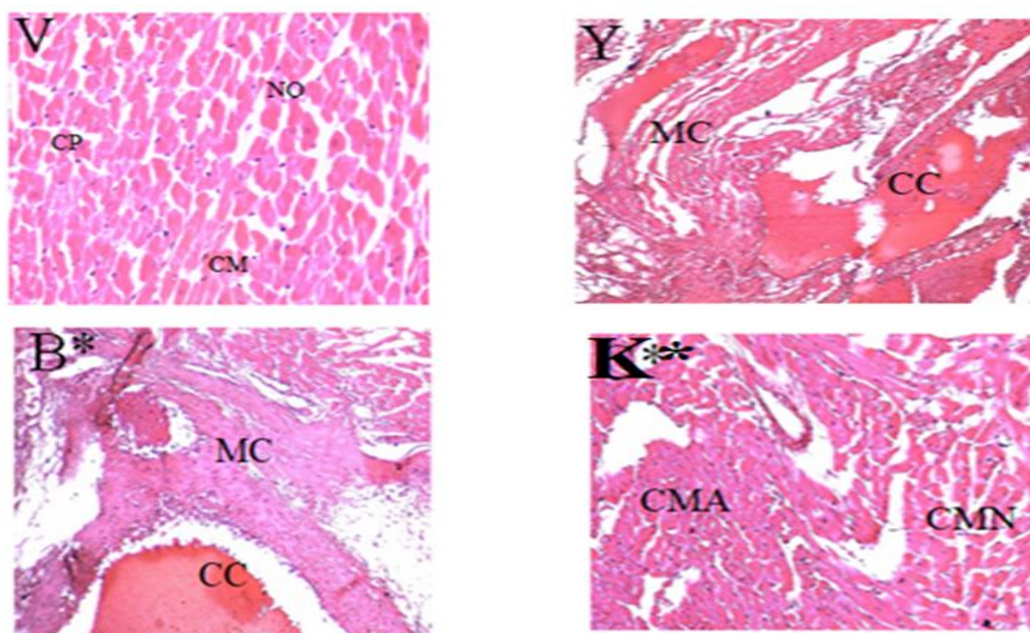


Figure 4: Histological section of the heart

F3: Fraction from *D. velutinum* leaves ; **V:** Control heart; **Y:** Heart of rats treated with gentamicin (80 mg/kg) ; **B*:** Heart of rats treated with gentamicin + fraction F3 (25 mg/kg) ; **K*:** Heart of rats treated with gentamicin + fraction F3 (50 mg/kg) ; **No:** Nucleus ; **CM:** Myocardial cells ; **CP:** Cytoplasm ; **MC:** Cardiac muscle ; **CMN:** Normal myocardial cells ; **CMA:** Atrophied myocardial cells ; **CC:** Cardiac congestion ; **Staining:** Hematoxylin-Eosin x40.

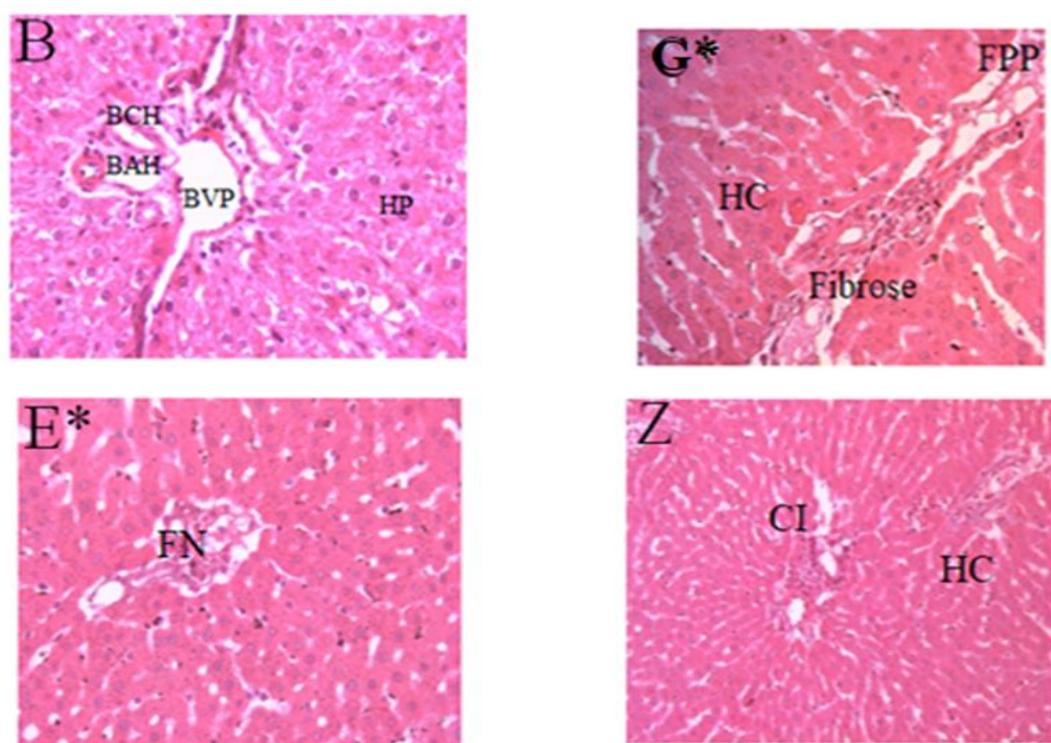


Figure 5: Histological section of the liver

F3: Fraction from *D. velutinum* leaves ; **B:** Control liver ; **Z:** Liver of rats treated with gentamicin (80 mg/kg) ; **G*:** Liver of rats treated with gentamicin + fraction F3 (25 mg/kg) ; **E*:** Liver of rats treated with gentamicin + fraction F3 (50 mg/kg) ; **BCH:** Hepatic duct branch ; **BAH:** Hepatic artery branch ; **BVH:** Hepatic vein branch ; **HC:** Hepatocytes ; **CI:** Inflammatory cells ; **FPP:** Periportal fibrosis ; **FN:** Normal liver ; **Staining:** Hematoxylin-Eosin x40.

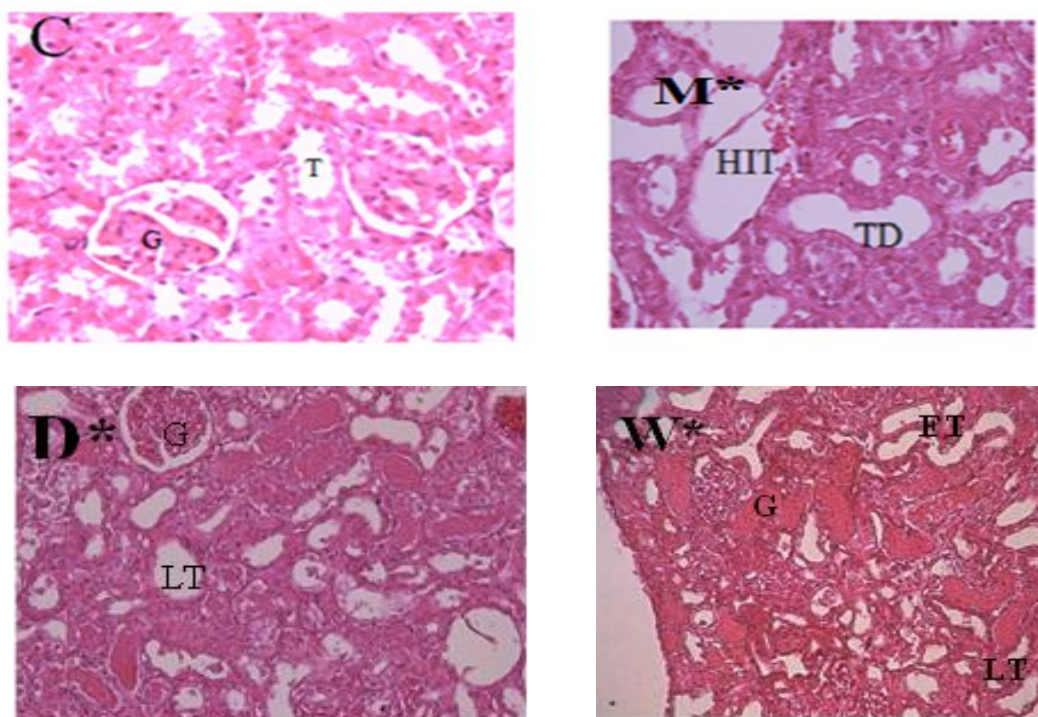


Figure 6: Histological section of the kidney

F3: Fraction from *D. velutinum* leaves; **C:** Control kidney; **M*:** Kidney of rats treated with gentamicin (80 mg/kg); **D*:** Kidney of rats treated with gentamicin + fraction F3 (25 mg/kg); **W*:** Kidney of rats treated with gentamicin + fraction F3 (50 mg/kg); **G:** Glomerulus; **T:** Tubule; **LT:** Tubule lumen; **ET:** Tubular space; **HIT:** Intratubular hemorrhage; **DT:** Tubular dystrophy; **Staining:** Hematoxylin-Eosin x40.

However, rats treated with 30% ethanol and fraction F3 from *D. velutinum* leaves (25 and 50 mg/kg) display a complete restructuring of the heart (Figures 7P and C*), with an architecture closely resembling that of the control rats.

2.3.2. Anatomopathological study of the liver

The treatment with 30% ethanol for 8 weeks led to vascular congestion in the portal branch and periportal necrosis in the liver of rats (Figure 8N). These findings contrast with the normal liver architecture observed in control rats (Figure 8B).

However, in rats treated with 30% ethanol + fraction F3 from *D. velutinum* (2.5 and 5 mg/kg), several normal hepatocytes were present, indicating functional recovery. Additionally, clear reappearance of the hepatic and periportal branches was observed (Figures 8Q and 8T*).

2.3.3. Anatomopathological study of the kidneys

The effects of 30% ethanol in rats treated for 8 weeks are shown in Figure 9. In rats receiving ethanol 30%, tubular dystrophy was observed (Figure 9F*).

However, in rats treated with ethanol 30% + fraction F3 from *D. velutinum* (25 and 50

mg/kg), the renal morphology appeared similar to that of control rats (Figure 9C).

Additionally, in treated rats (Figures 9O and 9R), a normal glomerular structure was observed, indicating partial restoration of renal function.

2.4. Thin-Layer Chromatography (TLC)

The chromatogram in Figure 10 presents the chromatographic profiles of fraction F3, obtained from the leaves of *D. velutinum*. This profile, after spraying the plate with Neu reagent and visualizing at 366 nm, reveals different streaks that indicate the presence of several chemical families.

The orange-yellow fluorescences with frontal ratios of 0.15, 0.28, and 0.58 are distinctly visible in fraction F3 and could be attributed to the derivatives of a flavonol ortho-dihydroxylated at positions 3' and 4' in the flavonol cycle (Figure 10).

The blue to bluish-white fluorescences, at ratios 0.72 and 0.85, may correspond to phenolic acid derivatives. The light-yellow compounds, with frontal ratios of 0.84 and 0.87, are characteristic of quercetin and myricetin derivatives.

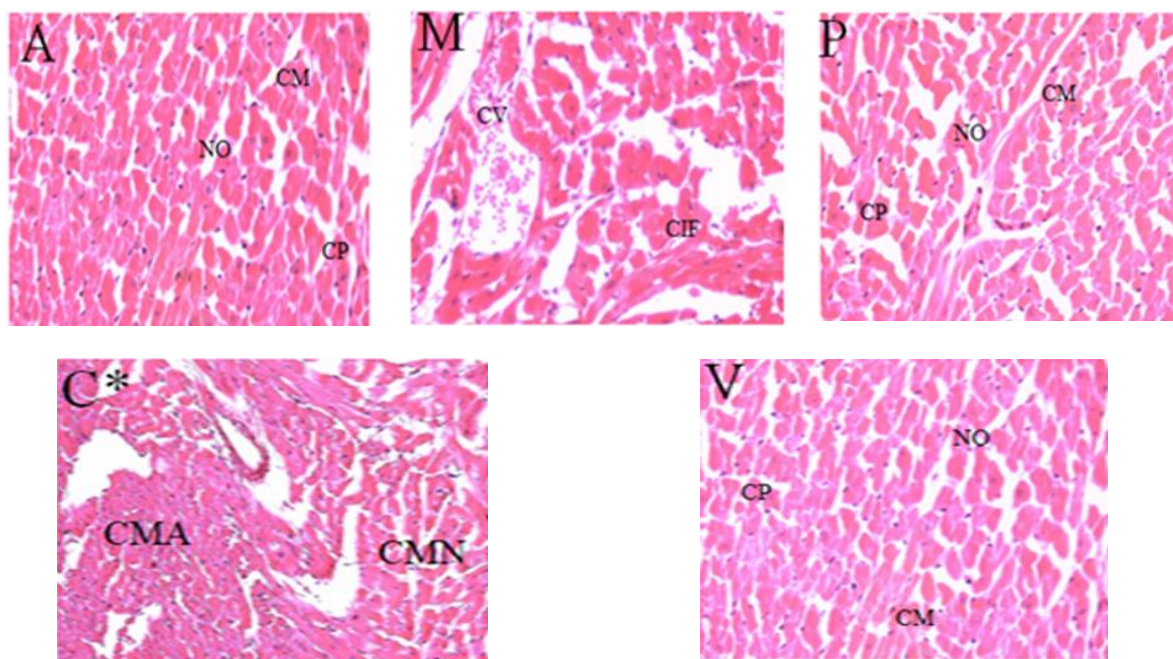


Figure 7: Histological section of the heart

F3: Fraction from *D. velutinum* leaves; A: Control heart; M: Heart of rats treated with 30% ethanol; P: Heart of rats treated with 30% ethanol + fraction F3 (25 mg/kg); C*: Heart of rats treated with 30% ethanol + fraction F3 (50 mg/kg); V: Heart of rats treated with 30% ethanol + Legalon (70 mg/kg); No: Nucleus; CM: Myocardial cells; CP: Cytoplasm; CV: Vascular congestion; CIF: Inflammatory cells; Staining: Hematoxylin-Eosin x40

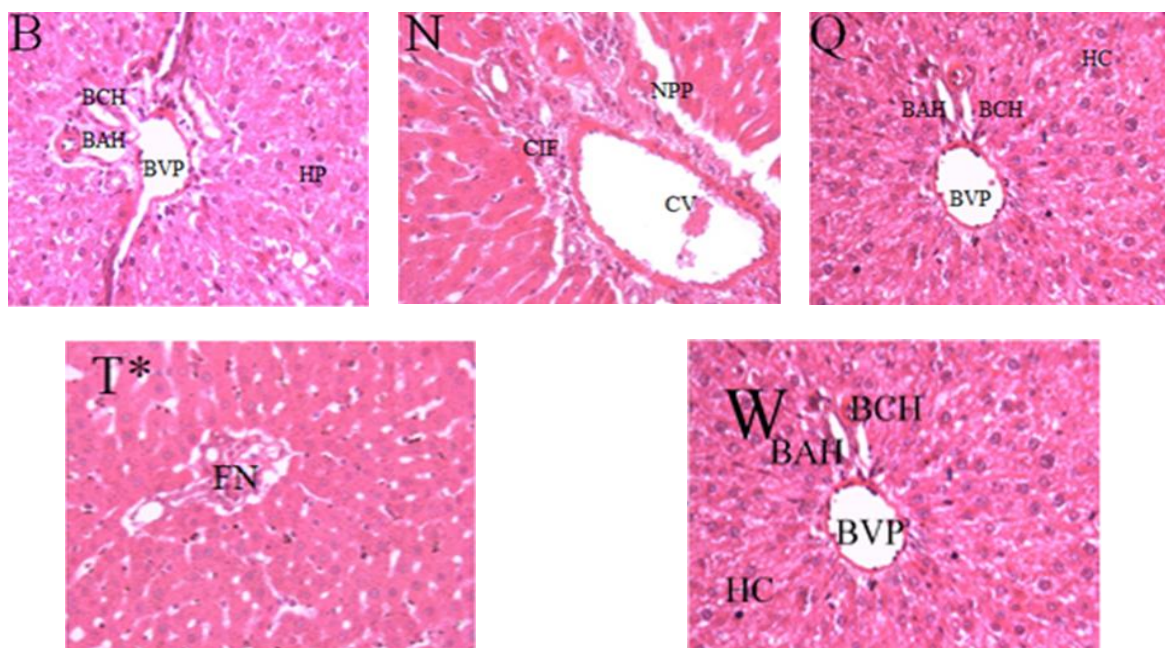


Figure 8: Histological section of the liver

F3: Fraction from *D. velutinum* leaves; B: Control liver; N: Liver of rats treated with 30 % ethanol; Q: Liver of rats treated with 30% ethanol + fraction F3 (25 mg/kg); T*: Liver of rats treated with 30 % ethanol + fraction F3 (50 mg/kg); W: Liver of rats treated with 30 % ethanol + Legalon (70 mg/kg); BCH: Hepatic duct branch; BAH : Hepatic artery branch; BVP: Hepatic vein branch; HC: Hepatocytes; CIF: Inflammatory cells; CV: Vascular congestion; FN: Normal liver; Staining: Hematoxylin-Eosin x40.

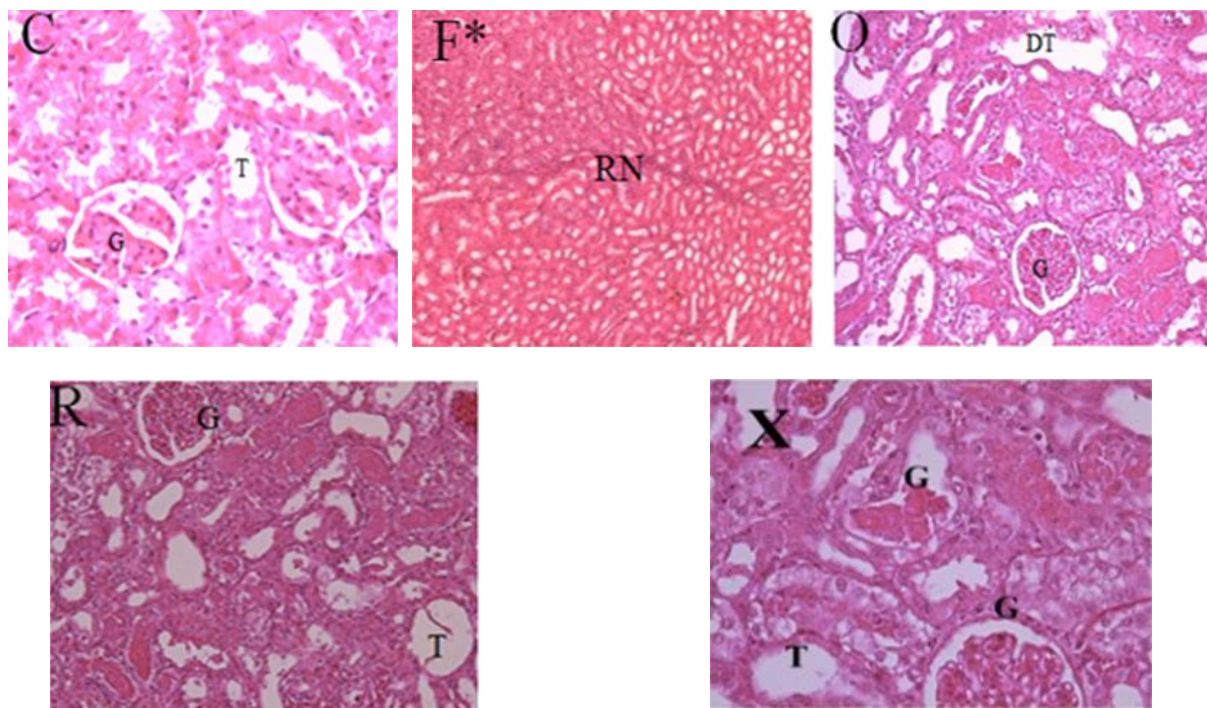


Figure 9: Histological Section of the Kidney

F3: Fraction from *D. velutinum* leaves ; **C:** Control kidney ; **O:** Kidney of rats treated with 30 % ethanol ; **F*:** Kidney of rats treated with 30 % ethanol + fraction F3 (25 mg/kg) ; **R:** Kidney of rats treated with 30% ethanol + fraction F3 (50 mg/kg) ; **X:** Kidney of rats treated with 30% ethanol + Legalon (70 mg/kg) ; **G:** Glomerulus ; **T:** Tubule ; **TN:** Normal renal structure ; **Staining:** Hematoxylin-Eosin x40.

2.5. Thin-Layer Chromatography (TLC)

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derivatives of a flavonol ortho-dihydroxylated at positions 3' and 4' in the flavonol cycle (Figure 10).

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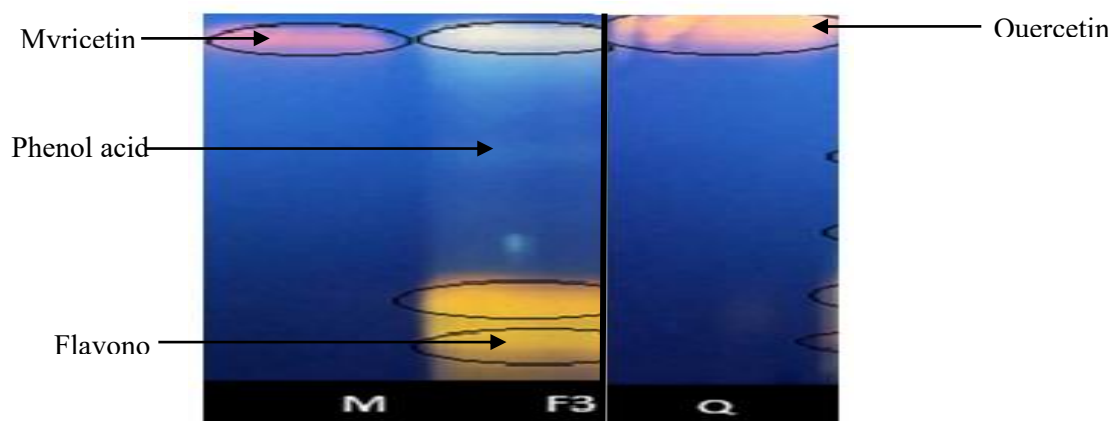


Figure 10: Thin-Layer Chromatography of the Extract and Fractions from *D. velutinum* Leaves
Reference compounds were analyzed using the following conditions : **Eluent:** Ethyl acetate/Formic acid/Water (9.5/0.25/0.25) ; **Revealing agent:** Neu reagent ; **Observation:** UV light at 366 nm ; **Reference compounds:** Q (Quercetin) and M (Myricetin) ; **Extract:** Hydroethanolic extract (EHE).

2.6. Anti-Radical Activity on Thin-Layer Chromatography

The anti-radical activity of fraction F3 was demonstrated using thin-layer chromatography (TLC). After spraying the plate with DPPH (2,2-Diphenyl-1-picrylhydrazyl), streaks on a violet background appeared on fraction F3, indicating the reduction of the free radical DPPH into a

more stable compound. This reaction confirms the presence of strong anti-radical activity on TLC.

This activity is particularly pronounced for fraction F3, and after one hour of observation, the reduction of the free radical continues progressively.

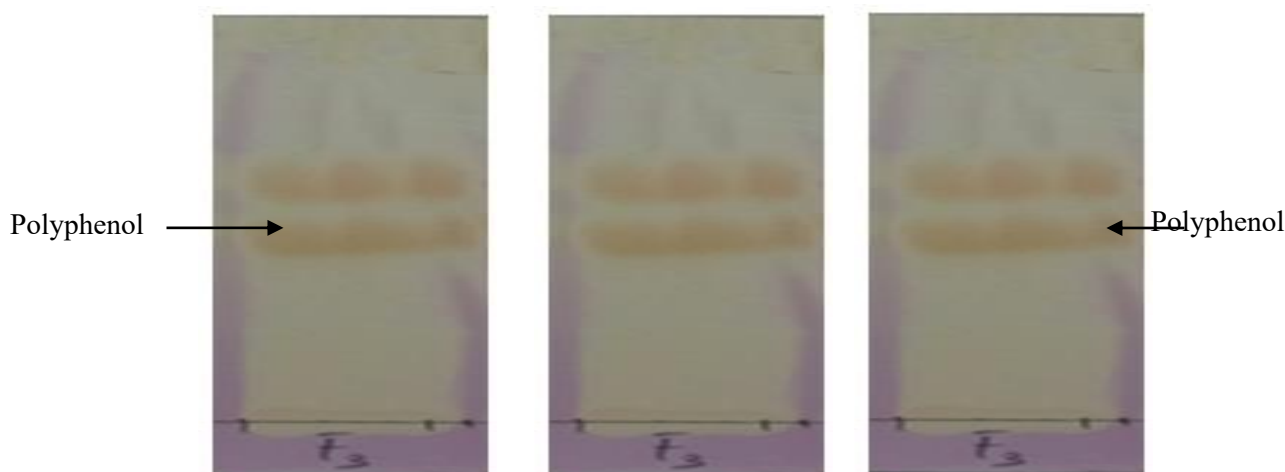


Figure 11: Anti-Radical Activity of the fraction from *D. velutinum* leaves
Eluent: Ethyl acetate/Formic acid/Water (8/1/1) ; **Revealing agent:** DPPH ; **Observation:** Daylight ;
F3: Fraction, observed until complete elimination of the radical.

3. Discussion

The administration of paracetamol (500 mg/kg, orally) for 14 days induced the formation of abnormal myocardial cell bundles and multiple inflammatory foci in the hearts of rats. These results suggest that paracetamol significantly influences cardiac function, which could partly explain the abnormal heart morphology and the quality of biopsies observed in histopathological sections.

Moreover, the presence of inflammatory cells and periportal necrosis observed in the liver of rats treated with paracetamol (500 mg/kg) could explain its adverse effects on hepatic function. It is well known that daily administration of paracetamol disrupts the liver's structural network (Makin & Williams, 2000).

The chain of inflammatory cells followed by atrophy of the renal tubule lumen, observed in the kidneys treated with paracetamol, could also partly explain paracetamol's nephrotoxic effects. In contrast, rats treated with fraction F3 from *D. velutinum* leaves (25 and 50 mg/kg) along with paracetamol (500 mg/kg) showed an improvement in the general architecture of the heart, liver, and kidney, suggesting a reversible

effect on the anomalies induced by paracetamol during treatment.

These results indicate that fraction F3 from *D. velutinum* leaves may exert a protective effect on vital organs. Similar findings have been obtained with the aqueous extract of *Ziziphus spina-christi* leaves (Al-Ghamdi *et al.*, 2019). The presence of cardiac congestion and inflammatory cells, observed in the heart and liver of rats treated with gentamicin (80 mg/kg), could explain the hemorrhagic foci detected in these organs. Furthermore, intratubular hemorrhages and tubular dystrophy, observed in the same rats, could justify gentamicin's nephrotoxic effects. Additionally, rats treated simultaneously with fraction F3 (25 and 50 mg/kg) from *D. velutinum* leaves and gentamicin (80 mg/kg) showed a restructuring of vital organs, with an architecture resembling that of control rats.

These findings suggest a potential protective effect on vital organs, likely due to the rich polyphenolic and flavonoid composition of fraction F3. It is well established that polyphenols and flavonoids exert protective and anti-radical effects on vital organs (Ammar *et al.*, 2009).

The presence of vascular congestion and inflammatory cells, observed in the hearts of rats treated with 30% ethanol and distilled water for 8 weeks, may indicate physical damage induced by ethanol administration. Studies have reported that alcohol consumption disrupts tissue function (Emeline Birckel, 2011).

On the other hand, other studies suggest that high alcohol consumption is significantly associated with an increased risk of inflammation and myocardial infarction (Ronksley *et al.*, 2011; Nguyen & Ancion, 2019).

Similarly, the presence of several inflammatory cells in the liver suggests a necrotic attack on hepatocytes. The periportal necrosis, also observed, indicates damage to the portal vein. Several authors have demonstrated that alcohol consumption profoundly alters the liver's structural network (Mello *et al.*, 2008). The tubular dystrophy observed in rats treated with

30 % ethanol and distilled water confirms that ethanol 30 % has nephrotoxic effects (Boffetta, 2006). Daily administration of fraction F3 counteracts the necrotic effects induced by ethanol, suggesting that fraction F3 may exert a beneficial effect on vital organs.

Furthermore, this fraction may be capable of preventing oxidative damage to vital organs induced by paracetamol, gentamicin, and ethanol.

The yellow-orange compounds detected by thin-layer chromatography in fraction F3 from *D. velutinum* leaves could be attributed to polyphenolic and flavonoid structures (Andzi Barhé *et al.*, 2020). Polyphenols and flavonoids are known to have protective effects (Jassim & Naji, 2003).

The strong anti-radical activity exerted by fraction F3 against DPPH could justify its protective effects on vital organs.

Conclusion

The present study aimed to evaluate the protective effects of fraction F3, derived from the aqueous extract of *D. velutinum* leaves, on vital organs in rats. Daily administration of fraction F3 (25 and 50 mg/kg) successfully counteracted the necrotic effects induced by paracetamol (500 mg/kg), gentamicin (80 mg/kg), and 30 % ethanol. Thus, fraction F3 has protective effects

on vital organs at doses of 25 and 50 mg/kg in rats. Moreover, fraction F3 from *D. velutinum* leaves was found to be rich in polyphenolic and flavonoid compounds. It also exhibited strong anti-radical activity against DPPH.

The protective properties of this fraction justify the traditional use of *D. velutinum* in medicine for combating certain diseases.

Conflict of Interest

The authors declare that there is no conflict of interest regarding this study.

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