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Therapeutic Potential of Cannabis sativa Extract in Mitigating Diclofenac-Induced Renal Toxicity in Wistar RatsShehu, R.M.¹ and Kabir, I.M.^{1,2*}

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Abstract

Renal toxicity is known to be majorly caused by therapeutic drugs such as antibiotics and analgesics, whose frequent usage, especially in high doses causes oxidative stress and free radicals. Free radicals damage the mitochondrial function of the kidney by interacting with the lipid membranes and reducing the antioxidant mechanism through lipid peroxidation. Natural products are also used as therapeutic agents for treating various diseases due to their minimal side effects. There is evidence that Cannabis is a potent anti-inflammatory molecule that reduces the production of reactive oxygen and nitrogen species, thus resulting in reduced tissue injury. The present study aims to investigate the therapeutic potential of Cannabis sativa extract in diclofenac-induced kidney damage in Wistar rats. Twenty-four Wistar rats were randomly divided into six (6) groups. The control groups were administered either physiological saline, diclofenac or Cannabis sativa extract, while the test groups were induced with kidney injury using 25 mg/kg body weight of diclofenac, and subsequently treated with 1000 mg/kg body weight of Cannabis sativa for varying period (up to 3 weeks). The rats were sacrificed after the respective treatment period, blood samples were collected and analyzed for renal biomarkers (urea and creatinine) and sections of the kidneys were made to evaluate the therapeutic effect of the extract histologically. The animals treated for one and two weeks showed a slow therapeutic response, but a remarkable response in the form of a general decrease in inflammatory response and oedema in kidney tissue was observed in

those treated for three weeks. There was also a steady decrease in the elevated serum urea and creatinine levels with increasing treatment duration. The restoration of renal biomarkers almost close to the normal range and a decrease in inflammatory responses indicate the therapeutic effects of some essential phytochemicals in the Cannabis sativa extract. However, further studies are required to identify and isolate the effective compounds responsible for these effects.

Keywords: Kidney damage, Diclofenac, Cannabis sativa, Wister rats, Histology

Introduction

The kidneys have a vital role in renal clearance, maintenance of blood pressure, elimination of toxic products and formation of prostaglandins (Sembulingam and Sembulingam, 2013). Kidney disease has been recognized as a leading public health problem worldwide. The global estimated prevalence of chronic kidney disease is 13.4% and patients with end-stage kidney disease needing renal replacement therapy is estimated at between 4.902 and 7.083 million (Zhang *et al.*, 2019). Renal toxicity is known to be the major drug-induced toxicity that causes oxidative stress and free radicals. The action of free radicals damages mitochondrial function through their interaction with the lipid membranes, hence causing a reduction of the antioxidant mechanism by the process of lipid peroxidation. However, this can be prevented by eliminating the production of free radicals and by reducing the extent of lipid peroxidation (Fattori *et al.*, 2017).

Certain medications are known to cause renal injury due to frequent usage and high dosage. Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of pain and arthritis. NSAIDs are the common drugs commercially used and prescribed frequently in most countries (Park *et al.*, 2017).

Natural products are also used as therapeutic agents for the treatment of various diseases due to their minimal side effects. These herbal plants are known to possess a vital role in protecting the body from chemical toxicity by restoring the antioxidant reserve, thereby minimizing oxidative stress (Mostafa *et al.*, 2020).

Marijuana refers to the dried leaves, flowers, stems, and seeds of the flowering plant classified under the family of Cannabaceae with various species such as *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis* (Moore, 2020). The plant contains the mind-altering chemical called tetrahydrocannabinol (THC) and other similar compounds such as cannabidiol and cannabinoids (Docimo *et al.*, 2013).

There is evidence that *Cannabis* is a potent anti-inflammatory molecule, which also reduces the production of reactive oxygen and nitrogen species, thus resulting in reduced tissue injury (Garza-Cervant *et al.*, 2020). In this work, we studied the potential therapeutic effects of *Cannabis sativa* extract on diclofenac-induced kidney damage in Wistar rats.

Methods

Experimental animals and Ethical approval

Twenty-four (24) apparently healthy Wistar rats (weighing 100g-300g) were sheltered and fed on a standard pellet diet and water at the Animal house of the Department of Pharmacology, Bayero University, Kano. The animals were kept and maintained under standard conditions of humidity and temperature in accordance with the laboratory animal care principles and allowed to acclimatize for a week prior to the commencement of the study. Ethical approval was obtained from the Animal Care and Use Research Ethics Committee of Bayero University, Kano.

Diclofenac injection

Diclofenac injection (A product of Feccox Pharmacy and General Enterprises Limited Kano, with Batch no: 211249) was purchased and used in this study.

Plant material and identification

The marijuana leaves were procured from the Nigerian Drug Law Enforcement Agency (NDLEA), Kano State Headquarters, Nigeria. The procured sample was identified as *Cannabis sativa* at the Department of Plant Biology, Bayero University, Kano and registered with the Herbarium number DPB/BUK/HIF/01282.

Phytochemical analysis of the extract

The crude extract was subjected to phytochemical analysis for Tannins, Saponins, flavonoids, glycosides, steroids, terpenoids, and phenols. The protocols were adopted from a previous study (Bisong and Buckley, 2014).

Preparation of the treatment

The median effective dose (ED₅₀) was determined using the Weil's method (1952). After collection, the leaves were ground into fine powder with a mortar and pestle. Four hundred grams (400g) of the powder was soaked in 2 litres of ethanol for 7 days and filtered with Whatman's filter paper. The filtrate was evaporated in a hot air oven to obtain the crude extract. The treatment was prepared by dissolving 5g of the crude extract in 10 mL of distilled water and homogenizing with a pestle and mortar using acacia powder as a facilitating agent. This gave a final concentration of 500mg/mL, which was stored in a sterile brown bottle at 2-8°C.

Toxicity test for Cannabis extract

The median lethal dose (LD₅₀) was determined by adopting Lorke's Possessive Case (1983). The procedure involves grouping animals into three groups of three animals per group and administering increasing concentrations of the treatment. In the first phase, the first, second and third groups were administered 10, 100, and 1000 mg of the extract per body weight respectively. In the second phase, a rat from the first group was orally administered 1600 mg of the extract per body weight. A rat from the

second group was administered 2900 mg of the extract per body weight, and a rat from the third group was administered 5000 mg of the extract per body weight. All groups were observed after 24 hours. The second phase was initiated only if the lower dosage from the first phase did not show any lethal effect.

A 20% concentration of 5000 mg was chosen as the appropriate dose for this study to avoid toxicity due to long-term administration according to the Lorke's Possessive Case (1983).

Toxicity test for diclofenac injection

A toxicity test (LD50) for diclofenac was also conducted in two animal phases, each comprising three rats. In the first phase, the first group was administered 50 mg of diclofenac per body weight at 0 and 24 hours and observed for 72 hours. In the second phase, the same was done for the second group with 25 mg of diclofenac per body weight (Peter and Evan, 2018; Abiola *et al.*, 2019; Mostafa *et al.*, 2020).

Weight of the animals pre- and post-intervention
The weights of the animals were recorded before and after the intervention. The differences in the means of the weights of the animals from each group were calculated.

Experimental design and treatment protocol

After establishing the toxicity and inducing kidney damage, 24 Wistar rats were randomly selected and divided into 6 groups, each consisting of 4 rats. Group 1 was administered physiological saline for 7 days. Group 2 (Cannabis extract control group) were orally administered 1000 mg/kg body weight of Cannabis per day for 21 days only. Group 3 (diclofenac control group) were administered 25

mg/kg body weight of diclofenac once intraperitoneally. Group 4 animals were administered 25 mg/kg body weight of diclofenac once intraperitoneally and subsequently orally administered 1000 mg/day of Cannabis extract for 7 days. Group 5 were intraperitoneally administered 25mg/kg body weight of diclofenac once and subsequently orally administered 1000 mg/day for 14 days of Cannabis extract. Finally, group 6 were intraperitoneally administered 25 mg/kg body weight of diclofenac once, and then orally administered 1000 mg/day for 21 days of Cannabis extract.

Animal sacrifice, sample collection and processing

All animals were sacrificed by cervical dislocation 24 hours after the final treatment. Blood samples were collected from the retro-orbital plexus into plain containers, and the animals were dissected to remove the kidneys. The harvested kidneys were preserved in 10% neutral buffered formalin immediately (Peter and Evan, 2018; Mostafa *et al.*, 2020).

The tissues were processed and stained using the Haematoxylin and Eosin staining method (Fischer *et al.*, 2008), and the slides were examined microscopically. Blood samples were spun and the collected serum was analyzed for serum Urea and Creatinine using Berthelot's and Jaffe's methods respectively (Tanganelli *et al.*, 1982; Ochei and Kolhatkar, 2008).

Results

Phytochemical analysis of the C. sativa extract

Phytochemical test for various phytoconstituents indicates that only Alkaloids are not present in the crude extract of C. sativa (Table 1).

Table 1. Phytochemical properties of the extract

Phytoconstituent	Test method	Result
Tannins	Ferric chloride test	+
Saponin	Frothing test	+
Flavonoid	Ammonium hydroxide test	+
Cardiac glycosides	Kella-Killiani's test	+
Anthraquinones	Chloroform	+
Steroids	Salkowski's test	+
Terpenoids	Salkowski's test	+
Alkaloids	Dragendroff's test	-

Toxic/Lethal Effects of the Diclofenac and *C. sativa* on the study animals

The Lethal dose (LD₅₀) of the treatments was determined in two phases for each treatment. For *C. sativa*, none of the doses caused any sign of toxicity or mortality, and 20% of the highest concentration was adopted as the effective dosage according to the Lorke's Possessive Case (1983). For Diclofenac, two out of the three animals in phase 1 died within 3 days when administered 50 mg/kg body weight, while all the 3 animals in phase 2 survived the 3 days testing period and half the dosage was used. Therefore, 25 mg/kg body weight of Diclofenac was used as the effective dose in this study (Table 2)

Table 2: Lethal effects of the treatments on the study Animals

<i>C. sativa</i> Dosage (mg/kg)	Mortality	Diclofenac Dosage (mg/kg)	Mortality
	Phase 1		
	(N=9)		(N=3)
10	-	50	1
100	-	50	-
1000	-	50	1
	Phase 2		
	(N=3)		(N=3)
1600	-	25	-
2900	-	25	-
5000	-	25	-

Post-intervention effects of *C. sativa* on the weight of the experimental animals

The average weight of the animals post-intervention increased in all groups except group 4, which lost an average of 5.5 g after being administered 25 mg/kg of Diclofenac once and 1000 mg/day of *C. sativa* for 7 days (Table 3).

Table 3: Post-intervention Effect of *C. sativa*. extract on weight (mean ± SEM)

	Weight (g)		
	Pre-intervention	Post-intervention	Difference
Group 1	192.00 ± 13.00	199.00 ± 29.00	16.00 ± 7.00
Group 2	184.50 ± 42.50	187.00 ± 69.00	26.50 ± 3.50
Group 3	142.25 ± 38.50	142.50 ± 41.00	2.50 ± 0.25
Group 4	244.00 ± 18.00	238.50 ± 20.50	-5.50 ± 2.50
Group 5	239.00 ± 0.00	246.00 ± 0.00	7.00 ± 0.00
Group 6	156.50 ± 49.50	158.50 ± 50.00	2.00 ± 0.50

Group 1 = Control (Normal saline), Group 2 = Control (*C. sativa*), Group 3 = Control (Diclofenac), Group 4 = Diclofenac once + *C. sativa* for 7days, Group 5 = Diclofenac once + *C. sativa* for 14days, Group 6 = Diclofenac once + *C. sativa* for 21days. SEM = (Standard Error of the Mean).

Effect of the treatments on renal dysfunction biomarkers

The mean values for urea and creatinine were determined for all the groups. A significant change was observed in the experimental groups compared to the reference ranges for Wister rats of 43.26-51.16 mg/dL and 2.57-3.05 mg/dL for urea and creatinine respectively. Diclofenac-administered rats showed significant elevation in the serum level of urea and creatinine. Groups 1 and 2 which received only normal saline and

Cannabis extracts respectively did not show any impairment in renal biomarkers. There is a significant elevation of serum renal biomarkers in Group 3 (diclofenac control group) by about 88% and 109% for urea and creatinine respectively. The groups that received *C. sativa* treatment showed a steady decrease in urea and creatinine levels with increasing treatment period, indicating the gradual healing of the induced kidney damage.

Table 4: Renal dysfunction biomarkers among the study groups

Group	Parameters		<i>p-value</i> *
	Urea (mg/dL) (Mean ± SEM)	Creatinine (mg/dL)	
1	47.37 ± 1.15	2.83 ± 0.45	0.50
2	48.56 ± 0.45	2.66 ± 0.35	0.92
3	89.10 ± 8.12	5.90 ± 0.10	0.93
4	75.81 ± 5.61	6.23 ± 0.90	0.86
5	67.15 ± 0.00	3.81 ± 0.00	—
6	56.78 ± 1.76	3.25 ± 0.15	0.12
Reference range	47.21 ± 3.95	2.81 ± 0.24	

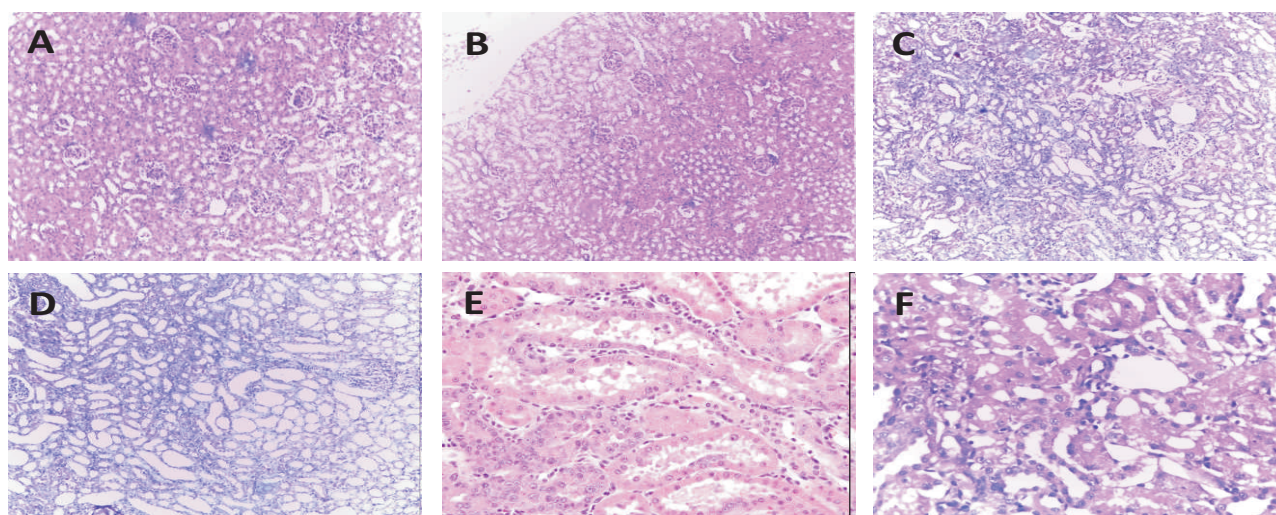
*Duncan's multiple range test.

Effect of *C. sativa* treatment on Diclofenac-induced kidney changes

Table 5: Histological findings on the therapeutic effect of *C. sativa* on the study animals

Group	Glomeruli	Tubules	Blood vessels	Interstitium
1	No inflammation No expansion No Necrosis	No inflammation No expansion, No Necrosis	No vascular necrosis	No oedema
2	No inflammation No expansion No Necrosis	No inflammation No expansion No Necrosis	No vascular necrosis	No oedema
3	Glomerular expansion and inflammation	Necrosis and Eosinophilic feature	No vascular necrosis	There is oedema
4	Glomerular expansion and inflammation	Necrosis and Eosinophilic feature	No vascular necrosis	There is oedema
5	Glomerular Necrosis and inflammation	Necrosis	No vascular necrosis	There is oedema
6	Glomerular expansion with no inflammation	Necrosis	No vascular necrosis	No oedema

Figure 1: Sections of kidneys from control and treatment groups indicating varying histological findings. A. Control (normal saline) group showing normal kidney histology, B. Control (*C. sativa*) group showing normal kidney histology, C. Control (Diclofenac) group showing glomerular expansion and inflammatory cells, D. Tubular expansion, necrosis and interstitial oedema after 7 days of treatment with *C. sativa*, E. Inflammation, tubular and glomerular necrosis, with no oedema after 14 days of treatment with *C. sativa*, F. Tubular and glomerular necrosis with no inflammation and no oedema after 21 days of treatment with *C. sativa*. (H and E $\times 100$ magnification: A-D, $\times 400$ magnification: E&F).



Discussion

In this study, two (2) experimental substances (diclofenac injection and *Cannabis* extract) have been tested on Wistar rats and their various effects were observed. A phytochemical analysis of the crude extract of *C. sativa* revealed some essential bioactive substances with nutritional values that give medicinal effects. The acute toxicity test of *Cannabis* extract was carried out to determine the adverse effects that may arise as a result of short-term exposure of the study animals to the extract within 24 hours. A review on prospective trial by Park *et al.* (2017) reported that medical marijuana use for pain control over a year did not result in significant changes in serum creatinine and urea. This claim is supported by the present study where 1000 mg/kg body weight of *C. sativa*. was administered every 24 hours over 21 days, and neither mortality nor kidney injury was observed in renal function tests and histological sections. The present study therefore suggests that *Cannabis* extract has very low toxicity since the lethal dose is higher than 5000 mg/kg body weight of the extract.

Rats treated with the extract in both the *C. sativa* control group and the test groups showed a significant increase in their appetite, leading to increased body weight. This observation complies with a study by Bhanushali *et al.* (2013) which revealed that a commonly reported side effect of *Cannabis* ingestion is the stimulation of appetite, particularly of palatable foods. Administration of either anandamide or 2-arachidonoylglycerol (synthetic analogues of Cannabinoid) also stimulates eating behaviour with increase in body weight (Bhanushali *et al.*, 2013).

In this study, normal saline and *Cannabis* extract control groups showed no histological and biochemical changes associated with kidney damage, suggesting that the animals were healthy and not predisposed to other external factors.

Diclofenac-administered rats showed significant impairment of renal function as indicated by elevated serum urea and creatinine compared to the normal saline control group. This can be associated with the absence of elimination of metabolized products from the body, resulting in severe

disruption of tubular function. The diclofenac-administered rats showed significant renal tissue deterioration such as glomerular inflammation, interstitial oedema, and tubular necrosis, these findings are in similar with the findings reported by Prince (2018) and Mostafa *et al.* (2020).

The administration of 1000 mg/kg body weight of *Cannabis* extract per day to the test groups over a period of 21 days has resulted to a significant decrease in the levels of serum urea and creatinine compared to the control group that was administered with diclofenac only. The histology of the test groups showed little improvement in the renal tissue. However, some pathological features observed in the diclofenac control groups were also seen in the tissue sections of the test groups, except for a general decrease in inflammatory features following treatment, indicating a slow response to therapy. This finding is supported by a study which reported that there is no evidence that the natural form of cannabinoids has any adverse effect on kidney function. Rather, cannabinoids prevented cisplatin-induced nephrotoxicity in a mouse model by reducing oxidative stress (Pan *et al.*, 2009). Meanwhile, some synthetic forms of cannabinoids such as XLR-11 have been identified as being nephrotoxic possibly due to their effect on proximal tubules and mitochondrial function, kidney biopsy most often demonstrates acute nephritis (Bhanushali *et al.*, 2013).

Conclusion

The study has shown pathological effect of diclofenac injection after examination of the renal histology and by elevated serum level of urea and creatinine, which indicate renal tissue damage. With the subsequent intervention of *C. sativa*. extract for over three (3) weeks, there was significant decrease in the level of serum urea and creatinine. Histological examination of the test groups post treatment showed no response after first and second week, but a remarkable response was observed after three (3) weeks treatment with *C. sativa* extract. There was persistent but mild glomerular and tubular necrosis in the renal tissues, the positive effect was general decrease in inflammatory response and oedema in kidney tissue sections.

Therefore, the restoration of renal biomarkers

closer to the normal range together with a decrease in inflammatory responses are indications of response to treatment by some essential phytochemicals present in the *C. sativa* extract known to have medicinal effects. Further studies are needed to evaluate the specific active compound(s) present in the plant and their mode of action.

Conflict of interest: The authors declare that they have no competing interests.

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