GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Biochemical activity of an herb *Commenlina diffusa* in curing sleeping sickness in Bida area

Ajawobu Oghale Innocent [1,*], Modozie Uchenna Benjamin [1], Dike Adaku Gloria [2] and Abdullahi Mann [3]

[1] *Department of pharmaceutical and medicinal chemistry, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria.*
[2] *Department of Science Laboratory Technology, University of Nigeria, Nsukka, Enugu state, Nigeria.*
[3] *Department of Chemistry, Federal University of Technology, Minna, Niger state, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2022, 21(01), 089–096

Publication history: Received on 28 August 2022; revised on 04 October 2022; accepted on 07 October 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.21.1.0377

Abstract

A good number of modern drugs have been isolated from medicinal plants which have led to sudden increase in the number of herbal drugs of recent. *Commenlina diffusa* is a widely known annual climbing dayflower that distributes dispersedly with heavy branches and slowly grows along the soil. The LD_{50} and biochemical effect of the plant extract was studied using 24 rats where the rats were grouped into 3 groups in 8 cages (A-G). Cages A-C as drugged with extract, D-G as inoculated with trypanosomiasis parasite (infected blood) and H as the control, they were all fed normally. The study revealed that the crude extract is tolerable below 0.22mg/100ml extraction. The biochemical assay showed an increase in the hepatic phospholipid and total glucose in the drugged rats (1.2;2.0mg/ml) while the inoculated rats with infected blood showed a decrease in hepatic phospholipid and higher hepatic glucose (1.0;2.5mg/ml) when compared with the control (1.1;1.2mg/ml). Moreover, the blood serum glucose revealed decreased in the drugged (2.40mg/ml) and increase for the inoculated rats (3.50mg/ml) when compared with the control (3.40mg/ml). The growth rate (weight difference) for the drugged is lower when compared with the control whereas the inoculated showed no significant difference, this could be as a result of the presence of phytochemical constituents in the extract. The study summarily suggests that the drug (extract) worked to oppose the infection via the gluconeogenesis pathway whereas the inoculated have their glucose synthesis elevated.

Keywords: *Commenlina diffusa*; Biochemical; Trypanosomiasis; Extract; LD_{50}

1. Introduction

Plants are a source of large amount of compounds consisting of groups of biologically active compounds such as anti-inflammatory, anti-microbial and anti-plasmodic. A good number of modern drugs have been isolated from medicinal plants and this has led to sudden increase in the number of herbal drugs (13).According to WHO (World Health Organization), 80% 0f the world's population depends on herbal medicines as primary health care. It also declared that around 21,000 plant species has potential of being medicinally used (14).

The plant *Commelina diffusa* has been being in used as a medicinal plant for centuries in several places, it is a pantropical plant which is also widely known as climbing dayflower or spreading dayflower belonging to the dayflower family; Commelinnaceae and is an herbaceous plant (1).It is a creeping perennial herb with prostrate or erect stem up to 45cm high, rooting at the nodes and reproducing from seeds and vegetative from stem shoots. The stem is round, freshly and smooth with swollen nodes, the flowers are pale blue in colour having three petals and open only in the margins, the fruit is a five – seeded capsule 3mm long (3).

*Corresponding author: Ajawobu O.I
Department of Pharmaceutical and Medicinal Chemistry, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria.

Historically, this plant *Commelina diffusa* has been a part of the effective herb used by Nupe herbal practitioners in Bida area for treatment of diverse diseases especially sleeping sickness as they claimed. The Nupe name for the plant is "*Edingi-bata"* and they claim that in treating such illments the use the extract after crushing and soaking in water to make paste, After which about two to four sharp horizontal marks are made on the neck region of the infected person (their patient), their believe is that, that is where massive load of the parasite resides once a person is infected (2). To this cut, the paste is applied and tied with a piece of cloth for about 24 to 48 hours wherein according to them if the organism is present, it will come out through those spots as they drug makes them uncomfortable. Thereafter, the spot is untied and washed off with clean water. The patient is observe for about 3 hours until no more outburst of house fly like maggot where the parasite is present, then palm oil is rubbed on the spot to aid healing of the wound. The patient is then placed on drinking boiled paste for some days or weeks depending as an extrusion therapy for cleansing off every parasitic trace in-vivo. Now, their application could be biochemical explained that the choice of the neck region is evident because of the lymphatic glands along the neck region; parasitic saturation if present will be more there.

Sleeping sickness also known as trypanosomiasis is caused by trypanosome carried to man by bites of tsetse flies of either sex. The disease is naturally acquired by Africans only between 12^0N and 25^0S (8).

It is in view of this claims and curiosity to create scientific balance that inspired the study on this medicinal plant with aim at elucidating the potency (bioactivity) of the extract by administering it to albino rats specially infested with the infected blood of trypanosomiasis.

2. Material and methods

2.1. Experimental Animals

Twenty four (24) Albino rats (12 males and 12 females) with weights ranging from 52 to 100g, these were obtained from National Institute of Trypanosomiasis Research (NITR), Vom, Plateau State, Nigeria. Two of the rats were inoculated with trypanosome of the species T.brucei gotten from infected cattle in Lafia, Nasarrawa State. They were brought to the animal house of the Department of Science Laboratory Technology, Federal Polytechnic Bida, Niger State. They animals were allowed to acclimatize for 1 week, although seven were lost remaining seventeen.

Thereafter, the animals were assigned to into eight cages of 2 animals each labeled A-H and were maintained under standard conditions with unrestricted access to standard diet (grower's mesh) and distilled water.

2.2. Medicinal Plant Extract

The medicinal plant was obtained very close to a rice swamp in a mini forest behind Nasiha Clinic of Esozhi Quarters in Bida Local Government Area, Niger State, Nigeria. The leaves were identified by Dr. P.O Imeokparia (Agronomist /Research Officier, National Cereal Research Institute, Badeggi, Niger State). The leaves were sundried for two weeks in the laboratory and then blended with an electric blender to powder this was then percolated (soaked) in ethanol for 72hours 100g powder to 1L ethanol (1:10) in cold maceration. There after the mixture was filtered using the vacuum pump and Buchner funnel, then filtrate was allowed to concentrate by placing it in a hot water bath at 80^oC for 5 hours to evaporate the ethanol thus producing the gel-like paste (the crude alkaloid drug)

2.3. Phytochemical Assaying On the Extract

The phytochemical analysis was carried out following the procedure as reported by (9) to screen for alkaloid, tannins, cardiac glycoside and saponin.

2.4. Experimentation to Ascertain Net Weight Changes

The weights of the rats were taken before experimentation then after one week of acclimatization, their weights were taken daily for 10days.

2.5. Separation of Rats into Various Cages for the Experimentation

The rats were separated into eight different cages where three cages served the purpose of establishing the toxicity of the drug in which two rats were arrayed per cage, four served the purpose of those inoculated with the infected blood in same array as above and one served as control cage with three rats. They were arrayed as shown below:

Table 1 Distribution of animals/cage to various groups

Toxicity Estb.			Inoculated Animals				Control
A	B	C	D	E	F	G	H
2	2	2	2	2	2	2	3

2.6. Establishment of Toxicity of Crude Extract (Drug) On Rats (Ld_{50})

Toxicity of any material is an expression of that material which will be able to kill or injure a living organism. This can be measured by administering the material/drug through any of the following means- nose, mouth or inter peritoneal by injecting depending on animal type. The LD_{50} known as the lethal dosage is defined as the dose when applied will kill 50% of the individual of a named batch of experimental animal treated with a foreign compound. As shown in the table above the rats in cages A-C to which the drug (crude extract) was administered in various the concentrations 200mg/100ml, 300mg/100ml, 400mg/100ml inter peritoneal given them 0.2ml daily for 5 days observing them, thus the probit response was plotted against the concentration from which half the probit plot antilog equates the LD_{50} following the method as described by (10)

Table 2 Dosage –Response for LD_{50} plot

Dose	Log Dose	Response	% Response	Probit

2.7. V Biochemical Analysis

The determination of glucose concentration and total phospholipids concentration in the harvested livers of the animals was carried using method as described by (10).

2.8. Statistical Analysis of Data

The Analysis of Variance (ANOVA) method was employed to compare mean difference observed among the various groups. The results are therefore presented as mean SD (Standard deviation with the level of significance set at $p<0.05$.

3. Results and Interpretation of Data

3.1. Alkaloid Extraction

- 100g powdered dry plant leaves in 1000ml of ethanol
- Yield dissolved solute of 650ml and then
- Gel-like extract from evaporation yields
- 12g (crude drug).
- Thus;Ratio 12:100
- 1.2:10
- 1:10(i.e 1g extract/10g powdered plant).

3.2. Photochemical Screening

Table 3 Results of the phytochemical screening

	Chemical components	Aqueous extract
1.	Alkaloid	+
2.	Saponins	+
	(Frothing test)	
3.	Tannins	+
4	Cardiac glycosides	
	Cardenolides	+
	Aglycone	++

+ -Positive; ++ - Strongly positive

3.3. Net weight change comparison

Table 4 and table 5 show the average weight of each group and the time/weight difference, indicating growth rate curve for individual rat type given same type of diet for sixteen days.The growth rate of the other rats was slightly significantly different from the control ($p < 0.05$) as they were higher than the control.

Table 4 Results of the average weight of animal groupings/day

Rat Group	Average Weight of Rat Types per day						
Drugged	84.7	102.0	112.3	114.3	114.3	114.0	114.2
Control	72.3	90.7	102.7	104.0	105.7	106.7	107.7
Inoculated	80.8	102.4	125.8	125.5	125.3	124.1	124.0

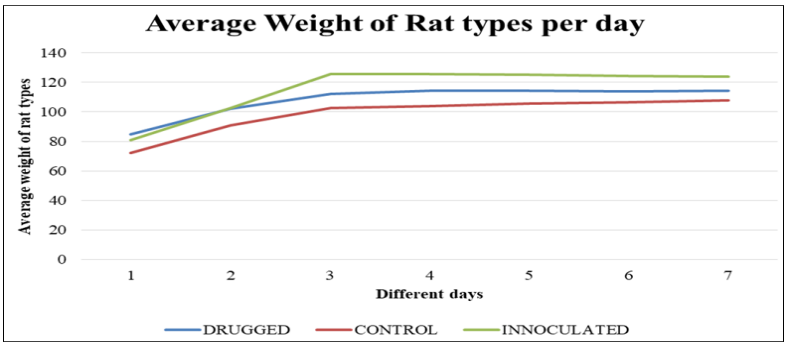

Figure 1 Plot of the average weight/day

Table 5 Time/weight difference

Rat Group	1st day	2nd day	3rd day	4th day	5th day	6th day
Drugged	17.3	27.6	29.6	29.6	29.3	29.5
Inoculated	21.6	45.0	44.7	44.5	43.3	43.2
Control	18.4	30.4	31.7	33.4	34.4	35.4

Time difference simply indicates the difference between a succeeding experimental day differences to the first day weight (example time difference between 2nd day and 1st day is, 102.4 - 80.8 = 21.6 under inoculated rat group.

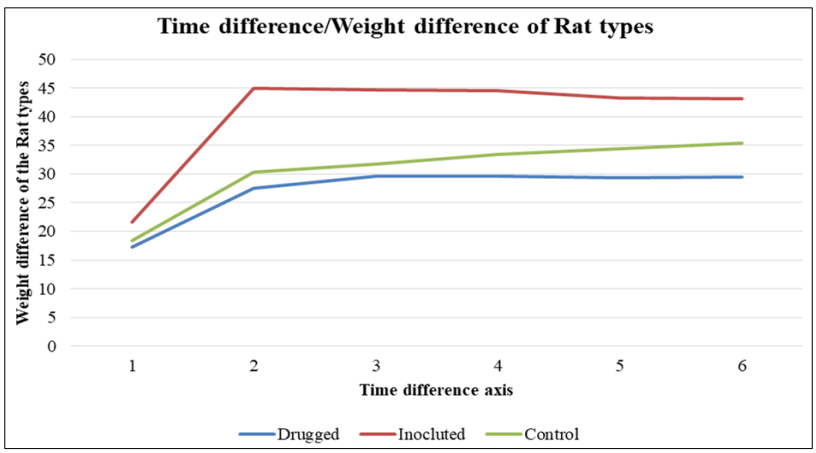

Figure 2 Plot of weight/time difference

3.4. Toxicity Establishment

Table 6 Dosage-Response Effect for LD_{50} Plot

Dose (g/100 ml)	Log dose	Response	% response	Probit from Tables
0.2	-0.7	0.0	0.0	2.9
0.3	-0.5	1.0	50.0	5.1
0.4	-0.4	2.0	100.0	6.4

From fig 3 below.it shows that the LD_{50} is -0.65 of which from the antilog will be equal to 0.22g/100ml extraction.

Table 7 Log. Dose/Probit relationship

Log Dose	Probit
-0.7	2.9
-0.5	5.1
-0.4	6.4

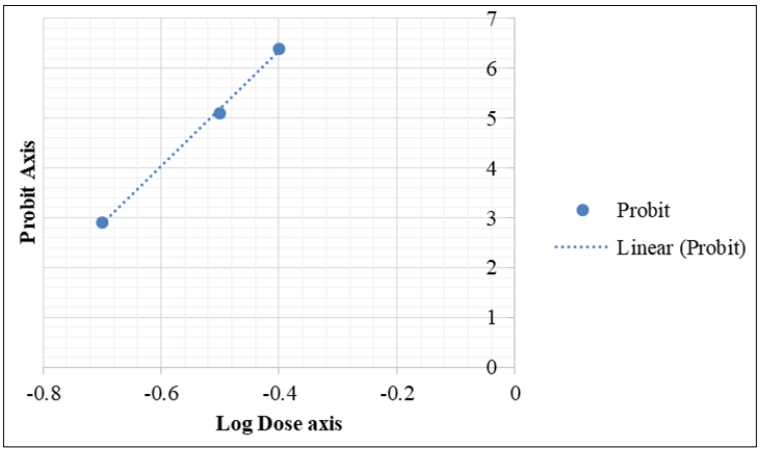

Figure 3 Lethal Dosage curve

3.5 Biochemical Analysis on Sacrificed Rats

Table 8 Body/liver weight comparison

Rat type	Body weight(g)	Liver weight(g)	Liver weight/ Body weight(g)
Control	83.0	7.2	0.09
Drugged			x0.07±0.008
1	145.0	9.4	0.06
2	124.0	8.4	0.07
3	84.0	6.3	0.08
Inoculated			x0.06±0.005
1	114.0	7.6	0.07
2	125.0	6.9	0.06
3	124.0	7.9	0.06

Table 9 Phospholipid Test

Rat Type	m(mg/ml)homogenate from graph (fig 3)	X(mg/ml) HomogenateCalculated	x(mg/g) Liver Weight
Control	0.0085	1.06	x 3. 18 + 0.00
Inoculated			
1.	0.0085	1.06	3.18
2.	0.0058	0.73	2.19
3.	0.0100	1.25	3.75
		x1.01 ±0.30	x 3.03 ±0.30
Drugged			
1.	0.0085	1.06	3.18
2.	0.0100	1.25	3.75
3.	0.0090	1.13	3.39
		x 1.15±0.08	x 3. 45 ±0.10

Table 10 Phospholipid standard curve plot

Concentration	Absorbance
0.01	0.15
0.02	0.26
0.03	0.44

Table 11 Glucose test (Liver homogenate)

Rat type	X(mg/ml)	Y(mg/g) Liver Weight	X/S.D(mg/ml) Homogenate
Control	1.20	3.60±0.00	1.20±0.00
Inoculated			
1	2.10	6.90±0.30	2.30±0.30
2	2.72	„	„
3	2.00	„	„
Drugged			
1	2.00	5.40±0.30	1.80±0.30
2	2.10	„	„
3	1.40	„	„

Table 12 Glucose test (Blood serum)

Rat type	X(mg/ml)	Y(mg/100mlserum)
Control	3.40	340.00
Inoculated	3.50	350.00
Drugged	2.40	240.00

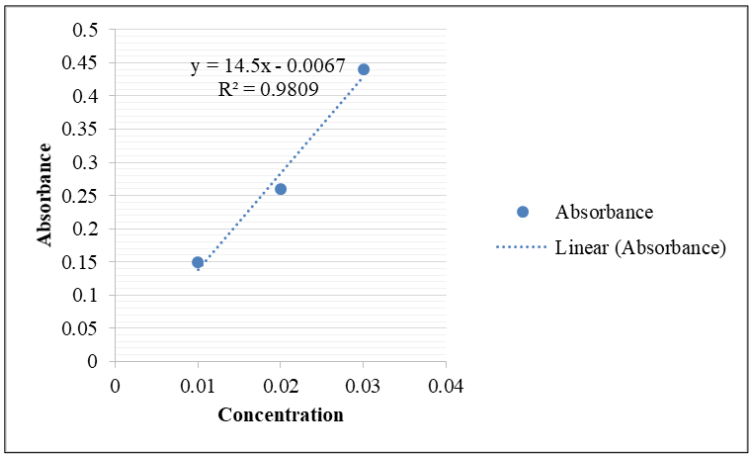

Figure 4 Plot of Phospholipid standard curve

4. Discussion

The result reveals that for every 10g of the plant leaves about 1g extract is gotten as against a higher yield of almost 3g as reported by (1) when methanol is used however, ethanol was used because of its easy digestibility and volatility as it is normally consumed.

The phytochemical screening on table 3 showed the presence of alkaloids, saponins, tannins and cardiac glycosides (cardenolides and aglycone), the alkaloid presence can be liable to the antioxidant property which also account for its anti-inflammatory property too and the saponins presence would have been responsible to the growth inhibition and also saponins are known to haemolyse erythrocytes though non- toxic to warm-blooded animals like ants, mammals etc when compared with the inoculated as also reported by (16;11). This fact could also be hypothetically inferred to as to why the rats didn't die even after applying the various dosage of the drug making it clear that the phytochemical constituents' presence have been eliciting noticeable effects.

The biochemical analysis in tables 8-12 revealed that the hepatic glucose level of the inoculated was quite higher than the control (2.3mg/ml:1,2mg/ml) however, that of the drugged is 1.8mg/ml whereas for the serum glucose inoculated was 3.5mg/ml, control 3.4mg/ml and drugged 2.4mg/ml. From all these, it shows that the inoculated increases glucose concentration in the liver and serum while the drugged increases the liver glucose slightly and reduced that of the serum glucose compared to the control (Table 11 and 12) since they all ate normal and same food, it is suggestive that while the inoculated elevates glucose synthesis, the drugged withdraws glucose from the blood thereby implicating gluconeogenesis pathway as a means of opposing the disease. Relative to the hepatic phospholipid assay on tables 9 and 10 showed a reverse with the drugged higher than the control and the inoculated lower than control thus showcasing that when the drug is used to treat the disease an equilibrium is brought which now stabilizes the phospholipid level to almost the control (Table 9). This was so reported also by (14).

The growth rate assay in table 4 showed a slight significant difference at $p<0.05$ (Table 4) which is suggestive that the drugged withdraws glucose from the blood stream apart from the liver and the slight drop in growth to be an anti-diabetic drug also.

The review work as reported by (1) shows that the plant has been used in fever, malaria, insect bite, bug bites rheumatoid arthritis, gonorrhea, influenza and bladder infection. They also reported pharmacological properties of the plant however, this aspect of trying to investigate a direct communal therapeutically practice has not be investigated where an alignment between orthodox and herbal traditional practice are brought to place.

5. Conclusion

The findings from this study strongly suggest that *Commenlina diffusa* leaf extract administered to the Wistar albino rats could reduce blood glucose since its mode of action is such that it increases hepatic phospholipid synthesis and withdrawing glucose from the blood thereby revering gluconeogenesis. Therefore, it could be inferred that the herbal medicine as used by the herbalist works by opposing any possible physiological imbalance that might be caused by the trypanosome. More so, this is suggestive of its being useful as an anti-diabetic drug.

Compliance with ethical standards

Acknowledgments

We are grateful to the Nigerian Institute of Trypanosomiasis Research, Vom, Plateau State Nigeria for providing us the experimental animal and the infected cow from which the inoculation to two of the rats was done. In same vein, our thanks go to Alhaji Baba Alhassan, the Trado-doctor that introduced us to the plant and also the Dept. of Science Laboratory Technology, Federal Polytechnic, Bida, Niger State for using their laboratory

Disclosure of conflict of interest

The authors declare no conflict of interest

Statement of ethical approval

The animals were given access to feed and water *ad libitum* and they were handled in compliance with the National Institute of Health Guidelines for the care of laboratory animals for research purposes (Pub. No.85-23, revised 1985)

References

[1] Afroza AP, Rubalat A, Asef F, Zaira Z, Pritesh RD. Pharmacological Importance of *COMMELINA DIFFUSA* (COMMELINACEAE): A REVIEW. Int'l Journal of Life Sciences and Review. 2019.

[2] Abdullahi M, Mohammed G, Abdulkadir N. Medicinal and Economic Plants in Nupeland. Jube Evans Publications. 2003.

[3] Agayakwa CW, Akobundu IO. Handbook of West African Weeds. IITA publishing company, Ibadan.1987; 418-419.

[4] Akerele O. Nature's Medicine Bounty: don't throw it away. World Health Forum. 1993; 14(4):390-5.

[5] David LN, Micheal MC. Lipid Biosynthesis in Lehninger- Principles of Biochemistry, 4th Edition.2005; 808-813.

[6] Davis H. Tsetse Flies in Nigeria. Pg. 1-70. Oxford University Press, Ibadan. 1977.

[7] Edegere H, Ekjindu G, Olatunde D, Magaji Y. Human Trypanosomiasis, A Fresh Profile of a Debilitating Disease in Nigeria by Serodiagnosis. Nigeria Journal of Science.1989; 23 Nos.1,2: 45-46. STAN, Lagos.

[8] Edwards IA, Bonchier AD, Christopher RW. Principles and Practice of Medicine. Longman Publishers. 1987; 779-780.

[9] Igweh AC. Some Phytochemical Analysis and Methodology. Nigerian Institute of Trypanosomiasis Research, (NITR) Vom, Plateau State. 1994.

[10] Igweh AC, Mary O, Onyekwelu NA, Osuei H. Guidelines and Instructions for Staff in Biochemistry and Immunology Departments, NITR, Kaduna/VomCentres. 1996.

[11] Martindale. The Extrapharmacopia, 31st Edition, Pharmaceutical Press, London. 2005.

[12] Meir Y. Sleeping Sickness in Encyclopedia of Science and Technology. 6th Edition.Mcgraw Hill Book Company, New York. 1987; 16: 467.

[13] Oluwagbenga S. Effect of Drying Methods on the Antimicrobial Properties of *Cassia alata*, *Commelinadiffusa* and *Borreriaverticillata* extracts. The Egyptian Journal of Experimental Biology. 2017; 13(1):1.

[14] Sule OJ, Arhoghroem,Erigbali P. Nephron-protective and hepto-protective property of *Commelinadiffusa* leaf extract in doxorubicin-induced albino rats. World. J. of Pharmacy Pharm.Sci. 2017; 6(10):51-62.

[15] Temitope MO, Damilola SA, Chukwuemelie ZU. Antioxidant Capacity of *Moringaoleifera* seed oil against CCL_4-induced Hepatocellular Lipid Peroxidation in Wistar Albino Rats. 2014.

[16] Wu SJ, Tsai JY, Chang SP, Lin DI, Wang SS, Huang SN, Ng LT. Supercritical Carbon dioxide Extract Exhibits Enhanced Antioxidant and Anti-inflammatory Activities of *Physalisperuviana:* Journal of Ethnopharmacology. 2006; 108(3):407-413.