Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

DOI: http://dx.doi.org/10.18203/2319-2003.ijbcp20164753

Original Research Article

Bioactivity and toxicity of Bridelia micrantha, Chenopodium ambrosoides and Ocimum americanum plant extracts

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Received: 08 November 2016
Accepted: 09 December 2016

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ABSTRACT

Background: *Bridelia micrantha, Chenopodium ambrosoides* and *Ocimum americanum* plant species are commonly used in traditional medicine for a number of ailments. The extracts of these plants have been shown to have anti-schistosomal activity suggesting that they could be used for the development of new chemical entities (NCEs) for the treatment of schistosomiasis. However there is limited knowledge on their toxicological profile and their use in traditional medicine may not be a satisfactory safety indication.
Methods: In this study the extracts were first screened for bioactivity using brine shrimp lethality test for the determination of LC50 followed by rodent acute toxicity and 28 day subchronic studies.
Results: *B. micrantha* water extract with a LC50 of 77µg/ml was deemed toxic while *C. ambrosoides* methanol and water extracts were moderately toxic with LC50 of 104.63µg/ml and 696.44µg/ml respectively. *O. americanum* hexane and water extracts toxicity varied from moderate to slightly toxic with LC50 of 887.59µg/ml and 2254.60µg/ml respectively. *C. ambrosoides* and *O. americanum* water extracts which were preferentially selected for subsequent studies were found to have mild to no irritation to rodent eyes and skin. Moreover, the aminotransferases AST and ALT which were used to detect liver injury suggested negligible effect.
Conclusions: This therefore confirms that *C. ambrosoides* and *O. americanum* water extracts are safe for clinical use with *O. americanum* water extract having a slight edge.

Keywords: Antihelminthic, Schistosomiasis, Toxicity

INTRODUCTION

Schistosomiasis is among the most common debilitating human disease caused by several species of the digenean blood trematode of the genus *Schistosoma*.[1] Infection is estimated at 200 million people with 120 million being asymptomatic, 20 million presenting with severe disease while 600 million are at risk of infection globally.[2] Annual mortality in Africa alone is estimated at 280,000 people and global burden is estimated at 4.5 million DALYs.[3,4]

Natural products especially of plant origin have proven to be reliable sources of therapies and new chemical entities

(NCEs) in medical history.[5-8] It is therefore not surprising that drugs used to treat 87% of categorised human diseases have a natural products origin and the period between 2000 to 2005 had 23 new drugs with other NCEs still undergoing trials.[7,9] Given the potential challenges of having praziquantel as the only commercially available anti-schistosomal drug such as emergence of resistance, it is crucial that studies aimed at identifying NCEs which will complement it be undertaken.[10-14]

Bridellia micrantha, *Chenopodium ambrosoides* and *Ocimum americanum* have been used as remedy for a variety of ailments including as antihelminthic applications.[15-17] In our separate studies and as reported by others, these plants have been shown to have antischistosomal properties.[18,19] However negligible toxicological studies have been undertaken to determine if the plants' bioactive molecules are safe particularly if intent is for clinical use.[20] OECD guidelines further elaborate that it is important to undertake these studies so that a decision to adopt products for clinical use or not can be determined.[21-23]

In this study brine shrimp (*Artemia salina*) lethality test was undertaken to determine LC_{50} followed by acute toxicity experiments which included eye irritation, dermal irritation and skin sensitization. Finally subchronic (28 day) study was performed to determine if the extracts had adverse effect on albino rats' livers. This study was performed at University of Nairobi's Department of Public Health, Pharmacology and Toxicology laboratory. Ethical approval for use of the animals was obtained from Institute of Primate Research's Institutional Review Committee (IRC).

METHODS

Brine shrimp bioassay

This assay was performed as described by Sorgeloos.[24] Briefly, 1g of *Artemia salina* eggs were hatched by incubation at room temperature in 3.3% artificial brine (Sera marine salt, Sera Company, Heinsberg, Germany) for 72 hrs. The plants extract solutions were constituted by dissolving 100 g of extract in 10 ml ddH_2O and topped up to 15 ml using artificial brine. This formed a stock solution of 1000 mg/ml for subsequent dilutions.

Serial dilutions were constituted in 15 ml test tubes by adding 500µl, 50µl and 5µl stock solution to 4.5 ml, 5 ml and 5 ml artificial brine respectively. Ten *Artemia salina* larval 1 stage larvae were added in each test tube and kept in light for 24 hrs followed by counting of surviving larvae. This test was performed in five replicates and the data analysed for determination of LC_{50}.

Acute dermal irritation test

This was performed as per OECD guidelines using three months old female Albino New Zealand White rabbits (*Oryctolagus cuniculus*).[25] After 7 days of acclimatization; they were shaved on two parts on the dorsal side with one abraded and the other left intact. The rabbits were maintained in cages with $20^oC \pm 3^oC$ and relative humidity at above 30% - but less than 70%-, with natural light. The animals were fed on pellets (Unga group Ltd, Nairobi, Kenya) with water *ad libitum*.

Water moistened paste of 0.5g of *C. ambrosoides* and *O. americanum* water extracts was applied on the two sites of each animal and covered with gauze held in place by a non-irritating tape. Care was taken to ensure the animal could not access the patch or inhale the applied extract. The animals were returned to the cages for the duration of the exposure (4 h) after which the gauze was removed and the area cleaned gently using water.

The animals were observed for signs of erythema and oedema at 24 h and 72 h post exposure period. As recommended in the OECD sequential testing strategy, the test was initially performed on one animal for either treatment followed by two replicates after confirming absence of severe effects and responses. The grading of erythema and oedema was based on a subjective score on a scale of 0 to 4 as described in OECD guidelines.[25]

Acute eye irritation study

Three months old female Albino New Zealand white rabbits (*Oryctolagus cuniculus*) were also used for this study as described in OECD guidelines.[26] Similarly to above section, the animals were allowed to acclimatize for a minimum of 7 days. Three animals were used for each of the two extracts and maintained in cages as described in the above section. The conjuctival sac was exposed to the extracts -0.5 g dissolved in water to form 0.1ml by sonication- by inserting 0.1 ml solution inside the lower eye lid then holding both eye lids together for a brief moment to allow for even distribution. The other eye which was not exposed to the extracts was used as a negative control. As articulated in above section, OECD sequential testing strategy was applied.

The eyes were observed at 24 h, 48 h, 72 h, 4 days and 7 days intervals post exposure and grading of ocular lesions; ulceration, opacity, necrosis and opaqueness on a scale of 0 to 4 as indicated in the OECD guideline.[26]

Skin sensitization test

This test was performed in ten week old female guinea pigs (*Cavia porcellus*) using a modified technique adapted from OECD guidelines.[27] The animals were maintained in cages at $20^oC \pm 3^oC$, with natural lighting, being fed on rodent pellets (Unga Ltd, Nairobi, Kenya) with water *ad libitum*. Three animals were used for each extract as well as control group.

Briefly, following a period of acclimatisation of not less than 7 days, the animals were shaved on their back on the

shoulder region taking care not to abrade the skin. They were then injected intradermally with 0.1 ml-0.5 g extract topped up to 0.1 ml with water, dissolved by sonication and then filtered through 0.45 µm nylon membrane (Acrodisc® Premium 25 mm syringe filter, Pall corporation, USA)- solution of each extract on the shaved area 3 times a week on alternate days for 3 weeks totalling 10 treatments. Control animals were injected with normal saline which served as sham treatment. Two weeks post exposure a challenge injection was administered and observation for clinical signs such as erythema, oedema, corrosion or general inflammation was made at 24 h, 7 days, 14 days, 28 days and 35 days.

The observations were graded according to Magnusson and Kligman for the evaluation of challenge patch test reactions with grading from 0-3.[27]

Subchronic toxicity (28 days)

This study was performed using albino rat (*Rattus albus*) for duration of 28 days with daily dosing as described in OECD guidelines.[28] Eight rats were used for each extract comprising of 4 individuals of either sex maintained in cages at 22°C ± 3°C and relative humidity at above 30% - but less than 70%-, with natural lighting. They were fed with standard rodent pellets (Unga feeds, Nairobi, Kenya) with water *ad libitum*.

Before the first dose was administered on day one, blood was collected from the orbital sinus and stored for later analysis as a baseline.[29] Oral doses were administered for 28 days at a concentration of 500 mg/kg and on the final day, blood was again collected from the orbital sinus and stored for final reading analysis then the animals were euthanized following ethical procedures. This was used as a preliminary study where if there was a noted effect, other haematological and pathological studies would have been undertaken. Biochemical analysis was performed on the collected blood using aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as indicators of liver cells damage. It is acceptable to measure these two parameters in drug development pre-clinical studies.[30-32]

Disposal of experimental animals

All animals used in these studies were euthanized by anaesthetizing using diethyl ether followed by incineration in specialized facilities.

RESULTS

Effects of the extracts on brine shrimp

The extracts of *B. micrantha, C. ambrosoides* and *O. americanum* at concentrations of up to 1000 µg/ml showed activity against brine shrimp and average mortality for each was determined. Finney's probit analysis was used for the determination of LC50 using Finney computer program and toxicity categorized as suggested by Rand.[33,34] The results are shown in Table 1.

Table 1: Brine shrimp mortality at various concentrations of extracts of *B. micrantha, C. ambrosoides* and *O. americanum*.

Concentration (µg/ml)	*B. micrantha* (water extract)	*C. ambrosoides* (methanol extract)	*C. ambrosoides* (water extract)	*O. americanum* (hexane extract)	*O. americanum* (water extract)
0	0	0	0	0	0
10	1.6	1.2	0.4	0.4	0
100	5.2	4.4	1.2	1.4	0
1000	9.2	9.6	6	4.2	3.4
LC_{50} (µg/ml)	77.24	104.63	696.44	887.59	2254.60
Toxicity categorization	Toxic	Moderately toxic	Moderately toxic	Moderately toxic	Slightly toxic

Table 2: Effects of dermal irritation of intact and abraded rabbit skins by aqueous extracts of *C. ambrosoides* and *O. americanum*.

		24 Hours				72 Hours			
		Intact		Abraded		Intact		Abraded	
		A	B	A	B	A	B	A	B
1	Erythema	0	0	1	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0
2	Erythema	0	0	1	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0
3	Erythema	0	0	1	0	0	0	0	0
	Edema	0	0	0	0	0	0	0	0

Key: A: *C. ambrosoides* water extract; B: *O. americanum* water extract

Skin irritant effects of extracts of C. ambrosoides and O. americanum

The results of the dermal irritation test showed that aqueous extract of *C. ambrosoides* was not significantly irritating to rabbit skin. There was however very slight erythema at 24 h post exposure on the abraded skin. Rabbits exposed to *O. americanum* water extracts on the other hand did not exhibit any effect. The results are represented in Table 2.

This suggests the biomolecules in *C. ambrosoides* responsible for the slight erythema cannot penetrate intact skin.

Acute eye irritation study

Mild changes were observed on the iris and some reddening where rabbits' eyes were exposed to *C. ambrosoides* water extract as shown in Table 3. This however dissipated by 48 h post exposure and appeared normal thereafter. There were no other observable effects on the cornea and conjunctiva. There was no observable effect for *O. americanum* water extract on the other hand as shown in Table 4.

Table 3: Rabbits eye irritation following exposure to *C. ambrosoides* water extract.

	CORNEA					IRIS					CONJUNCTIVAE				
	Opacity					**Changes**					**Chemosis**				
	24 h	48 h	72 h	4 Days	7 Days	24 h	48 h	72 h	4 Days	7 Days	24 h	48 h	72 h	4 Days	7 Days
1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	Area involved					**Redness**					**Discharge**				
	24 h	48 h	72 h	4 Days	7 Days	24 h	48 h	72 h	4 Days	7 Days	24 h	48 h	72 h	4 Days	7 Days
1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

Table 4: Rabbits eye irritation following exposure to *O. americanum* water extract.

	CORNEA					IRIS					CONJUNCTIVAE				
	Opacity					**Changes**					**Chemosis**				
	24 h	48 h	72 h	4 Days	7 Days	24 h	48 h	72 h	4 Days	7 Days	24 h	48 h	72 h	4 Days	7 Days
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Area involved					**Redness**					**Discharge**				
	24 h	48 h	72 h	4 Days	7 Days	24 h	48 h	72 h	4 Days	7 Days	24 h	48 h	72 h	4 Days	7 Days
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Skin sensitization

No visible changes were observed for the skin sensitization study for the two extracts as shown in Tables 5 and 6.

Subchronic toxicity

AST and ALT concentrations post exposure were categorised relative to means of initial concentration which was assumed to be the upper limit of the normal (ULN). These were categorised as proposed by Thapa and Walia as follows; severe (> 20 times ULN), moderate (3-20 times ULN) and mild (1-3 times ULN).

C. ambrosides, AST was 1.53 times for females and 1.52 for males which corresponds to mild category. ALT on the other hand was 0.96 times for females and 0.95 times for males which does not meet any of the categorization criteria suggesting there is no effect. The results are shown in greater detail in Table 7.

Table 5: Guinea pig skin sensitization following exposure to *C. ambrosoides* water extract.

	Reaction	24 h	7 Days	14 Days	28 Days	35 Days
1	Erythema	0	0	0	0	0
	Edema others	0	0	0	0	0
2	Erythema	0	0	0	0	0
	Edema others	0	0	0	0	0
3	Erythema	0	0	0	0	0
	Edema others	0	0	0	0	0

O. americanum AST was 1.14 times for females and 1.18 for males which also corresponds to the mild category. ALT on the other hand was 0.86 times for females and 0.80 times for males which does not meet any of the applied criteria suggesting there is no effect.

Table 6: Guinea pig skin sensitization following exposure to *O. americanum* water extract.

Replicates	Reaction	24 h	7 Days	14 Days	28 Days	35 Days
1	Erythema	0	0	0	0	0
	Edema others	0	0	0	0	0
2	Erythema	0	0	0	0	0
	Edema others	0	0	0	0	0
3	Erythema	0	0	0	0	0
	Edema others	0	0	0	0	0

Table 7: Albino rats sub chronic toxicity (28 days) following exposure to *C. ambrosoides* water extract.

		AST (iu)		ALT (iu)				
No	**Sex**	**Initial**	**Final**	**Initial**	**Final**	**Dose**	**Initial wt (gms)**	**Final wt (gms)**
1	F	134.00	122.40	67.43	65.60	500mg/kg	215.60	202.30
2	F	103.40	220.20	50.51	48.90	500mg/kg	220.40	203.60
3	F	106.20	218.10	54.60	52.70	500mg/kg	185.60	187.10
4	F	117.50	145.60	62.80	59.40	500mg/kg	204.60	193.50
Mean		115.28	176.58	58.84	56.65			
5	M	137.00	128.70	71.79	69.80	500mg/kg	192.40	180.40
6	M	102.60	221.90	53.56	51.75	500mg/kg	181.30	178.40
7	M	110.40	218.10	56.50	53.60	500mg/kg	162.10	154.20
8	M	120.30	150.10	62.30	58.40	500mg/kg	159.30	156.80
Mean		117.58	179.70	61.04	58.39			

Table 8: Albino rats subchronic toxicity (28 days) following exposure to *O. americanum* water extract.

		AST (iu)		ALT (iu)				
No	**Sex**	**Initial**	**Final**	**Initial**	**Final**	**Dose**	**Initial wt (gms)**	**Final wt (gms)**
1	F	78.20	101.10	54.25	48.90	500mg/kg	143.50	132.50
2	F	135.30	132.40	51.30	46.36	500mg/kg	140.60	120.70
3	F	86.20	101.30	46.24	42.44	500mg/kg	149.60	138.60
4	F	91.20	110.60	58.30	43.21	500mg/kg	138.90	129.00
Mean		97.73	111.35	52.52	45.23			
5	M	80.40	103.80	56.39	41.71	500mg/kg	122.50	110.60
6	M	138.80	154.90	56.75	43.22	500mg/kg	187.50	176.40
7	M	87.40	102.60	47.54	43.62	500mg/kg	125.20	118.60
8	M	93.40	112.80	58.10	47.50	500mg/kg	134.00	127.40
Mean		100.00	118.53	54.70	44.01			

These results therefore suggest the water extracts of *O. americanum* and *C. ambrosoides* have little to no effect on the liver and therefore relatively safe.

DISCUSSION

Due to the use of these plants in traditional medicine, one could assume that they are completely safe. However, studies have shown that there is a risk as such plants may have adverse effects despite their widespread use.[35,36] This therefore implies the importance to determine the

toxicological profile of extracts studied for their potential use in drug development.

In this study, brine shrimp toxicity was used as a screening test before other more elaborate studies could be performed. This led to the elimination of *B. micrantha* water extract which was found to be toxic at the same time allowing for the preferential selection of *O. americanum* and *C. ambrosoides*. The principle here was exclusion of any extracts that would otherwise lead to unnecessary discomfort in downstream rodent based studies.

C. ambrosoides water extract was observed to be a mild dermal irritant on the abraded skin at 24 h resolving by 48 h and remaining normal thereafter. There was also mild irritation observed on the iris accompanied with some redness. No sign of skin sensitization was however observed. *O. ambrosoides* water extract on the other hand was not found to be a dermal irritant, eye irritant and there were no observable changes for skin sensitization. These results suggest that both these extracts are relatively safe when exposed to eyes or skin with *O. americanum* water extract having a slight edge.

Having ruled out adverse reaction on topical application, it was important to study the effect on liver injury using aminotrasferases AST and ALT as indicators of hepatocellular necrosis.[37] Both extracts were found to have mild effect on the liver for female rats while for males, there was no observable elevation. This suggests the extracts have minimal to no effect on internal organs and the effect is sex dependent.

CONCLUSION

In conclusion, *C. ambrosoides* and *O. americanum* water extracts therefore have a desirable toxicological profile suggesting they can be used for development of new antischistosomal drug candidates that will be relatively safe for human use. While *C. ambrosoides* has biomolecules that can lead to slight irritation, the effect was deemed to be mild and therefore negligible.

AKNOWLEDGEMENTS

We are grateful to Stanley Mathenge and Nicodemus Muia for technical support in this study

This work would not be possible without the financial support of NACOSTI and DAAD.

Funding: Funding provided from NACOSTI and DAAD
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Osebe T, Mbaria J, Yole D, Odongo D, Nderitu J, Ochanda H. Bioactivity and toxicity of Bridelia micrantha, Chenopodium ambrosoides and Ocimum americanum plant extracts. Int J Basic Clin Pharmacol 2017;6:5-11.