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Cytotoxicity and Acute Oral Toxicity Study on Quassin and Fractions of *Quassia amara* Extract

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Abstract

Different fractions of *Quassia amara* and its bioactive compound quassin have been implicated in male reproductive toxicology. Toxicity results obtained from brine shrimp lethality test on chloroform, methanol and hexane fractions of *Quassia amara* gave LC_{50} value of 93.4569µg/ml, 172.0463µg/ml, and 46.1941µg/ml respectively. LC_{50} of quassin was 0.0000µg/ml. Acute oral toxicity was by OECD limit test procedure and methanol extract of *Quassia amara* showed no lethality up to 5000mg/kg. *Quassia amara* and quassin therefore has toxicity activity. Methanol fraction is comparatively least toxic to brine shrimp and also well tolerated by the systemic organs of experimental rats.

***Keywords*:** *Quassia amara*; quassin; toxicity; brine shrimp.

1.0 Introduction

Quassia amara is a 6-8 meters tall tree native to Suriname, Brazil, in South America and is naturally distributed in several tropical countries. It is a member of the *Simaroubaceae* family of plants, which are neither shrubs nor tress. It grows 4 to 6 meters in height. Its synonyms include suriname, amargo and bitterwood.

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Phytochemical screening has shown that *Q. amara* extract is majorly made up of compounds collectively called quassinoids. A minute percentage of canthinone alkaloids have also been identified. The major quassinoid isolated from *Q. amara* is called quassin [1].

Quassin is present in every part of *Q. amara* plant irrespective of the time of collection [2]. It has been used as flavorings in beverages and in baked foods [3]. Extract of *Q. amara*, a plant with strong antimalarial and antimicrobial activities was reported to have potent toxicity effect on male reproductive parameters in experimental rats and mice [4,5,6,7].

Its bioactive compound, quassin has been implicated as the antifertility agent in rats [5]. In continuation of these, recent reports from our laboratory suggest that this plant extract may have toxicity effect that is specific to the reproductive tissues. Its deleterious effects on total epididymal tissue protein and sperm capacitation were reported [8]. This further emphasizes the possibility of its use as a contraceptive agent as earlier suggested by Parveen *et al*. [6] and Faisal *et al*. [7].

Different solvents viz methanol [5,7], hexane [9] and chloroform [6] have been reported used for studies on *Q. amara* actions. In other to specifically target the reproductive tissues and avoid systemic toxicity, the fraction with the least toxic activity will be ideal. This study was therefore designed to determine the toxicity level of various fractions of *Q. amara* using brine shrimp lethality test. Acute oral toxicity study was then carried out on the least toxic fraction to observe whole animal toxicity.

2.0 Methodology

2.1 Brine shrimp cytotoxicity testing

The toxicity level of the quassin and fractions of *Q. amara* extract was conducted as described by Falope *et al.* [10] and Oloyede *et al.* [11]. Ten grams of *Artemia salina* (Brine shrimps egg) were introduced into 50ml of sea water in a hatching chamber and left under fluorescent light for 48hours at room temperature to hatch. The nauplii (harvested shrimps) were attracted to one side of the vials by the light source. The extract (0.02g) was then dissolved in 0.2ml of dimethyl sulphoxide (DMSO) and then diluted with 1.8ml of sea water to give a 2ml stock solution containing 10,000ppm of the extract.

Serial concentrations (10,000, 1,000 and 100µg/ml) of the extract was prepared in DMSO and sea water. Each of the extract concentrations (0.5ml) was then dispersed into series of test tubes, and this was followed by the addition of 4.5ml sea water and subsequently 10 larvae of brine shrimps to each tube. A concurrent control experiment set up was prepared, and had no extract. All experiments were conducted in triplicate. The set up was left for 24 hours after which surviving shrimps in each test tube was counted and recorded. The concentration for killing fifty percent of the larvae (LC_{50}) was calculated using computer Finney programme [11].

2.2 Acute oral toxicity study

Q. amara was reported to be well tolerated when administered daily (orally) to male rats for duration of 8 weeks at doses up to 2000mg/kg. On this basis, acute oral toxicity on whole animal was conducted using the OECD Limit test procedure (OECD, 2001). Nine male rats, divided into 3 groups of 3 animals each were fasted overnight. Single oral dose (2000mg/kg) of methanol extract of *Q. amara* was administered to the first group and 5000mg/kg to the second group. The third group was administered 5ml of distilled water (solvent) orally. Animals were then observed individually, for 2 hours for behavioral, neurological and autonomic profiles, then periodically over the next 72 hours and daily for 14 days, with special attention within the first 4 hours for any lethality, moribund state or death [12]. All procedures in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding principles in the care and Use of animals [13].

3.0 Results

3.1 Brine shrimp lethality test

The toxicity result of quassin and different fractions of *Q. amara* extract showed that it is toxic to brine shrimp larvae. LC_{50} was less than 300µg/ml in all fractions and zero in quassin treated larvae. Methanol fraction however had the highest LC_{50} (Table 1).

3.2 Acute oral toxicity test

After more than 14 days observatory period, there were neither deaths nor visible physical damage to the *Q. amara* treated rats. Animals were without any visible and identifiable side effects or mortality even at 5000mg/kg bw (Table 2).

Table 1: Brine shrimp lethality test of *Q. amara* extract and Quassin

Chloroform Fraction						
	Dead	Alive	Dead	Alive	Dead	Alive
10,000ppm	10	-	10	-	10	-
1.000ppm	9	1	9	1	10	-
100ppm	6	4	5	5	5	5
LC_{50} (µg/ml)	93.4569					
Methanol Fraction						
	Dead	Alive	Dead	Alive	Dead	Alive
10,000ppm	10	-	10	-	10	-
1.000ppm	10	-	7	3	7	3
100ppm	4	6	5	5	3	7
LC_{50} (µg/ml)	172.0463					
Hexane Fraction						

	Dead	Alive	Dead	Alive	Dead	Alive
10,000ppm	10	-	10	-	10	-
1.000ppm	10	-	8	2	10	-
100ppm	7	3	5	3	7	3
LC_{50} (µg/ml)	46.1941					
Quassin						
	Dead	Alive	Dead	Alive	Dead	Alive
10,000ppm	10	-	10	-	10	-
1,000ppm	10	-	10	-	10	-
100ppm	10	-	10	-	10	-
LC_{50} (µg/ml)	0.0000					

*LC_{50}- Lethal concentration for 50% of the larvae

Table 2: Acute oral toxicity on *Quassia amara* extract

Quassia amara	**Survival**	**Death**
2000mg/kg	100%	NIL
5000mg/kg	100%	NIL
Control (5ml distilled water)	100%	NIL

4.0 Discussion and Conclusion

Several beneficial effects of *Q. amara* plant have been reported in literature which includes as an antibiotic, antimalarial, antifungal and anti ulcerative agent [14,15]. Quassin, the major bioactive constituent of the plantis also used as flavorings and additives to beverages and baked foods. Reports of antifertility action of quassin in rats [5] however call for caution in the use and consumption of quassin in humans. More so, no information exists till date on absorption, distribution, metabolism or excretion of quassin [16].

Different solvents have hitherto been reported in literature for extracting the active constituents of *Q. amara* plant for reproductive toxicology and similar studies. Examples include use of methanol [5,7], hexane [9] and chloroform [6]. In this study, toxicity level of these various *Q. amara* fractions was determined using brine shrimp lethality test. Our results show that methanol extract, with

an LC_{50} of 172.0463µg/ml is comparatively least toxic. This is followed by chloroform, with an LC_{50} of 93.4569µg/ml. Hexane fraction is however most toxic of the fractions, as its calculated was LC_{50} 46.1941µg/ml. All brine shrimps larvae exposed to the pure compound quassin died, even at the lowest dose of 100ppm used in this study. Since all calculated LC_{50} was less that 300µg/ml, this indicates that *Q. amara* extract have toxicity activity [10,11]. Quassin however has a more potent toxicity activity than the crude extract.

Having observed that the methanol fraction has the least toxicity activity, acute oral toxicity study was then carried out on the methanol fraction to observe whole animal toxicity. Animals showed no sign of mortality after dosage with single limit test dose of 2000mg/kg and 5000mg/kg. All animals survived the 14 day study duration. Lack of toxicity or morbidity after acute oral administration of methanol fraction of *Q. amara* to rats suggests that the fraction is tolerated by the systemic organs.

In conclusion, it may be inferred from this study that methanol fraction of *Q. amara* is less toxic when compared with the chloroform and hexane fractions. The pure compound quassin is however very toxic to brine shrimps. Therefore, it is recommended that the methanol fraction be administered to experimental animals in future studies targeting specific organ toxicity. A classical example is toxicity studies specifically targeting the reproductive tissues, to achieve male contraceptive functions for which *Q. amara* is being proposed [6,7,8].

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