Safety evaluation of polysaccharides isolated from the water extract of *Argemone mexicana* L. (Papaveraceae) in *Drosophila melanogaster*

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**ABSTRACT**

Recently, the plant polysaccharides have attracted attention due to their important bioactivities. The literature has shown several pharmacological activities of *Argemone mexicana* extracts and its components but very few data on its polysaccharides. The current study aimed to evaluate the safety of polysaccharides from *A. mexicana*. Five polysaccharides [High molecular weight (polysaccharide fraction) of the water extract from *Argemone mexicana* 1 (HMAm1), HMAm2, HMAm3, HMAmA1, and HMAmA2] were fractionated from *A. mexicana* aerial parts by using accelerated solvent extractor procedure followed by ion exchange chromatography of the water decoction extract. The safety assay was carried out using *Drosophila melanogaster* exposed to the polysaccharides at 12.5 and 25 µg/ml for 72 hours against a negative control (1% DMSO). After the exposure period, the survival rate and the locomotor capacity of flies were determined. At the end of 72 hours of treatment, all polysaccharide fractions at both doses presented a survival percent of more than 94%. In addition, these polysaccharide fractions affected very little the locomotor performance of the flies. At both doses, HMAm2 presented the highest safety for the flies, while HMAm3 was the least. These findings revealed that polysaccharides from *A. mexicana* are nontoxic.

**INTRODUCTION**

Polysaccharides are polymers which are present in several organisms, including tissues of seeds, stems, and leaves of herbal plants, body fluids of animals, shells of crustaceans and insects, cell walls, and extra cellular fluids of bacteria, yeast, and fungi (Singh et al., 2012). Scientists give a huge attention to polysaccharides from the medicinal plants due to their significant bioactivities, such as anti-tumor, antioxidant, anticoagulant, antidiabetic, radioprotection, anti-viral, hypolipidemic, and immunomodulatory activities. No side effects reported yet for plant polysaccharides (Xie et al., 2016). There is a need to perform further investigations on these polymers.

*Argemone mexicana*, an annual herb belonging to family Papaveraceae, is commonly found in tropical and subtropical regions of the world (Husna and Reddy, 2017). Native of tropical America, this species is known as Mexican poppy or Mexican prickly poppy (Sharanappa and Vidyasagar, 2014). It is a prickly and annual herb with 1.2 m in high. In Mali, the plant is locally known in Bamanan as “Bozobo” or “Nienidjeni”.

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Nigeria, *A. mexicana* is locally known in Hausa as “Kaki ruwan Allah,” “Karanko” or “Kwarkwaro” (Ibrahim et al., 2016). In different parts of the world, *A. mexicana* is used to treat many diseases, including tumors, warts, skin diseases, inflammations, rheumatism, jaundice, leprosy, microbial infections, and malaria (Brahmachari et al., 2013). Earlier authors reported the juice from leaves and the latex of *A. mexicana* mixed with lemon juice are used in the treatment of malaria (Bapna et al., 2015). *Argemone mexicana* is one of the most effective plants used traditionally to treat uncomplicated malaria in Mali (Diallo et al., 2006). In addition, the *in vitro* antimalarial activity of *A. mexicana* was carried out at the Swiss Tropical Institute in Basel in collaboration with the Department of Traditional Medicine of Mali. These authors revealed that the 50% inhibitory concentration values of the decoction, maceration, dichloromethane (DCM), and methanol extracts against the chloroquine-resistant K1 strain of *P. falciparum* were 5.89, 6.22, 1.22, and 1.00 µg/ml, respectively (Willcox et al., 2007). A randomized controlled trial comparing *A. mexicana* aerial parts decoction with artesunate-amodiaquine (standard first-line treatment) in the management of uncomplicated malaria revealed that 89% of patients recovered clinically with the plant decoction versus 95% for artesunate-amodiaquine (Graz et al., 2010). These last authors found also that there are no significant differences between the groups in most of the outcome measures, and both treatments were well tolerated. In addition, there was no deterioration of severe malaria in patients >5 years but 1.9% deterioration in children ≤5 years were observed in clinical trials (Molokhia et al., 2011). On the other hand, analgesic, antispasmodic, possibly hallucinogenic, and sedative properties make this weed plant a medicine traditionally used to treat malaria, warts, cold sore, skin disease, and itches (Motilal et al., 2017). Wound healing property, vasoconstrictor and vasorelaxant effects, antimicrobial, fungitoxic, anti-stress and anti-allergic, analgesic, anti-inflammatory, antifertility, nematicidal, larvicidal, molluscicidal, hepatoprotective, cytotoxic, anticancer, anti-HIV, antioxidant, anti-diabetic, and antimalarial activities of the plant extracts were reported (Sharanappa and Vidyasagar, 2011). Besides pharmaceutical efficacies, toxicity effects (acute toxicity, epidemic dropsy, hepatotoxicity, etc.) of certain parts of the plant related to some alkaloids (e.g., sanguinarine) are also narrated (Brahmachari et al., 2013).

Beyond alkaloids, this plant species contains other chemical classes, such as terpenoids, steroids, carbohydrates, long-chain aliphatic alcohols and carboxylic acids, amino acids, flavonoids, and other phenolics (Brahmachari et al., 2013).

In Mali, the results from sub-chronic toxicity studies of the decoction of *A. mexicana* in rats revealed the safety of the aerial parts of the plant (Sanogo et al., 2008). The Department of traditional medicine in Mali produces *A. mexicana* as a standardized phytemedicine for the management of malaria. The three protoberberine alkaloids (berberine, protopine, and allocryptopine) present in *A. mexicana* showed similar high antimalarial activities (Willcox et al., 2011). But, the absorption of berberine is poor in some animal models, while the pharmacokinetic of protopine and allocryptopine has not been studied yet in humans. In addition, earlier antimalarial tests using the freeze dried *A. mexicana* decoction were unsuccessful both in mouse and in rat models using *Plasmodium berghei* and *Plasmodium chabaudi*, respectively (Willcox et al., 2011). Water soluble compounds having immunomodulatory properties may be involved in such antimalarial activity. Very few previous studies have been reported on polysaccharides from *A. mexicana* decoction. Among many insects *Drosophila melanogaster* and *Artemia salina* are widely used as model organism to perform the pharmacological and toxicological activities of chemical compounds and natural products (Siddique et al., 2005; Zemolin et al., 2014). *Drosophila melanogaster* has been extensively studied as a decisive model in biology about a century ago. The fly shares several basic biological, biochemical, neurological, and physiological similarities with mammals. About 75% of human disease-causing genes have functional homolog in *D. melanogaster* (Abolaji et al., 2013). In addition, the fly can well be kept at low cost in the laboratory, and it has been recommended as an alternative model to vertebrate usage. Therefore, its genetically importance coupled with its low cost and its high sensitivity to toxic substances make it a suitable model (Bezerra et al., 2017). The aim of this study is to evaluate the safety of the polysaccharides from *A. mexicana* using *D. melanogaster*.

**MATERIALS AND METHODS**

**Plant material**

The aerial parts of *A. mexicana* were collected at Blendio in Mali, September 2014, and identified by a taxonomist of the Department of Traditional Medicine (DMT came from its french name which is Département de Médecine Traditionnelle), Bamako, Mali. Then, a voucher specimen (2948/DMT) is deposited at the herbarium of the DMT. The aerial parts were dried, pulverized to a fine powder by a mechanical grinder, and kept for further investigations.

**Extraction and fractionation of polysaccharide fractions**

Sample was extracted using an Accelerated Solvent Extractor (Dionex ASE350, Sunnyvale, CA, USA). Powdered aerial parts, (500 g) were weighed and mixed with diatomaceous earth (125 g). Due to the capacity of the stainless steel cells, 316.5 g of the mixture only were packed in eight stainless steel cells of 100 ml two times. Each set was performed using 1,500 psi, with 5 minutes heating, 5 minutes static time, and a 250 seconds purge for a total of three cycles.

To remove low molecular (LM) weight compounds, the plant material was pre-extracted three times with dichloromethane at 40°C, followed by 96% ethanol (EtOH) at 60°C. Then, the residue was extracted three times with 50% ethanol-water (EtOH-H<sub>2</sub>O) at 50°C, followed by distilled water at 100°C. The water extracts from the two extraction sets were gathered and submitted to the ultrafiltration (cut off 5,000 Da) to separate the LM weight part to the high molecular (HM) weight. The LM weight was concentrated then lyophilized, while the HM weight was dialyzed at cut-off 3,500 Da. The dialyzed HM was then fractionated by an ion exchange chromatography as described by (Zou et al., 2014) with slight modifications. Five acidic fractions called High molecular weight (polysaccharide fraction) of the
water extract from Argemone mexicana 1 (HMAm1), HMAm2, HMAm3, HMAmA1, and HMAmA2 were isolated and kept to determine their effect on the flies.

**Drosophila melanogaster stock and culture**

*Drosophila melanogaster* (Harwich strain) fruit flies were obtained from the fly laboratory of Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria. The flies were maintained at 25°C ± 1°C and 60%–70% relative humidity and were kept with a 12 hours dark/light cycle. The basal culture medium or diet contained 100.0 g of corn flour, 20.0 g of yeast, 16.0 g of agar, and 1700.0 ml of distilled water and methyl paraben (1 g in 5 ml of ethanol) were added to prevent bacteria contamination as described by Chen *et al.* (2018) with some modifications. All experiments were performed with the same strain.

**Polysaccharides exposure and survival rate**

*Drosophila melanogaster* (both genders), 1–3 days old, were divided into 15 groups of 30 flies each. Five polysaccharide fractions were investigated for their effect on the flies. Among the 15 groups, two doses of each fraction were compared to the control consisting of 1% dimethyl sulfoxide (DMSO). Polysaccharide fractions were dissolved in 1% DMSO: Water (1:99) to get 12.5 and 25 µg/ml as plant treatments. Test substances (supplemented diets) were prepared by adding 1% DMSO, 12.5 and 25 µg/ml for each polysaccharide fraction in the basal diet to obtain final concentrations of 10% of these treatments. Hence, for the control the diet (9 g) was mixed with 1% DMSO (1 g), while plant polysaccharides were obtained by adding 1 g of each concentration to 9 g of the basal diet in a beaker. Ten grammes of each supplemented diet were poured into the bottom of a vial and dried for the test.

Exposure of flies to polysaccharide fractions was performed as described by Coutinho *et al.* (2017) with some modifications. For each fraction, three vials received the following supplemented diets: control (culture medium+ 1% DMSO) and 12.5 and 25 µg/ml, respectively. Then, 30 adult flies (males and females of 1–3 days old) anesthetized on ice were placed in each of the 15 vials containing the supplemented diet. Survival readings of the flies have done at 3, 6, 12, 24, 48, and 72 hours. At the end of each experiment, all tested doses were compared to the mean of the control. The results are presented as percentage (%) of live flies (mean ± standard error of mean) obtained from three independent experiments.

**Locomotor assay**

The locomotor capacity was evaluated by using the negative geotaxis behavior as described by Abolaji *et al.* (2014) with some modifications.

Briefly, 150 adult flies (4–6 days old: both genders) were used per experiment. After 72 hours exposure to the control (1% DMSO) and the different doses (12.5 and 25 µg/ml) of each polysaccharide fractions, 10 flies from each supplemented diet vial were transferred randomly in an empty vial prior to place them in a vertical glass column (length: 15 cm; diameter: 2.5 cm) for the locomotor capacity. Then, flies were gently tapped to the bottom of the glass column, and the number of flies that climbed up to the 6 cm mark of the glass column in 6 seconds as well as those that remained below this mark after this time, were recorded. For each batch of 10 flies, this procedure was repeated three times at 1-minute interval. The scores represent the mean of the number of flies at the top \( (n_{top}) \) expressed as a percentage of the total number of flies \( (n_{tot}) \) recorded in three independent experiments.

**RESULTS AND DISCUSSION**

**Polysaccharides exposure and survival rate**

After 72 hours of the exposure, there was no significant change in the survival rate of flies (Fig. 1). The finding showed that the survival percent was higher than 94%. The exposure period was too short to kill 50% of flies with each polysaccharide fraction; however, this period is enough to evaluate the toxicity of antimalarial agent in *D. melanogaster*. In our laboratory conditions, the highest dose (25 µg/ml in 10 g of diet) of most of the fractions kept the flies alive at least for 48 hours. HMAm2 showed the highest safety with 100% survival observed for both doses 25 and 12.5 µg/ml in 10 g of diet. There is a slight difference at 12.5 µg/ml between HMAm1 and HMAm2. On other hand, the two concentrations of HMAm3 affected more flies. The control group and those treated with 12.5 µg/ml showed more cases of mortality than 25 µg/ml, this could explain the protection in dose dependent manner of the tested polysaccharide fractions. The mixture of polysaccharides from *A. mexicana* increased the survival time in mice with tumoral cells of lymphocytic leukemia P-388 and sarcoma 37 (Gil *et al.*, 2005). To our knowledge, few studies have been performed on the polysaccharides from *A. mexicana*. Previous investigations of the *Argemone* decoction administrated per oral revealed that the leaves of *A. mexicana* are nontoxic in rats (Sanogo *et al.*, 2008) and the phytomedicine made with this plant species is safe and tolerable in human (Willcox *et al.*, 2011). However, the plant extract administrated intraperitoneally in mice (18–25 g and averagely aged between 4 and 6 weeks) exhibited an acute toxicity in mice, where the LD50 value was 400 mg/kg body weight (Ibrahim and Ibrahim, 2009). This toxicity could be due to the mode of administration.

**Locomotor assay**

Locomotor performance of exposed flies to the control (1% DMSO) and polysaccharide fractions at 25 and 12.5 µg/ml for 72 hours are presented in Figure 2. The capacity of flies to climb after polysaccharide exposure is a good factor to support the safety of these natural substances. In overall, climbing behavior of treated flies with polysaccharides were not really affected when compared with the control group. At 12.5 µg/ml, the following samples HMAm1 and HMAm2 exhibited the highest capacity to cross the 6 cm mark in 6 seconds when compared to the control (Fig. 2). The climbing percent at 12.5 µg/ml were 93.3, 90.0, and 86.6 for HMAm1, HMAm2, and HMAmA2, respectively. For 25µg/ml, the highest climbing percent were 92.2%, 87.8%, and 84.5% for HMAmA1, HMAm2, and HMAm1, respectively. The lowest climbing percent at 12.5 µg/ml were 83.3% and 84.4% for HMAmA1 and HMAm3, respectively, while at 25 µg/ml HMAm3 and HMAmA2 presented the lowest climbing percent with 77.8% each. Overall, 12.5 µg/ml showed a higher protection of the flies than
Contrary to heat shock protein 70 (hsp70), one of the most expressed stress protein families was increased by Argemone seed oil at 1.0 µl/ml in D. melanogaster third-instar larvae (Mukhopadhyay et al., 2002).

CONCLUSION

The results collectively indicated that the polysaccharide fractions from A. mexicana are safe for D. melanogaster when the flies are exposed to different concentrations of these polysaccharides. These results support the innocuousness of the water extract from A. mexicana aerial parts used in the traditional medicine against various indications. Further biological investigations will be undertaken on these polysaccharide fractions to reveal their possible effect against malaria.
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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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