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ACUTE TOXICITY STUDIES AND ANTIDOTAL THERAPY OF ETHANOL EXTRACT OF *JATROPHA CURCAS* SEEDS IN EXPERIMENTAL ANIMALS

Onome T. Abiri[1], *Mohamed Samai[1], Ayesha Koker[1], Mohamed Bawoh[1] and Helen O. Kwanashie[2]

[1]Department of Pharmacology, Faculty of Basic Medical Sciences, College of Medicine and Allied Health Sciences, University of Sierra Leone, Freetown, Sierra Leone 2Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

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Abstract

In spite of the myriad of ethno medical uses and agro-feed potential of Jatropha curcas (*JC*) seeds and the potential for production of biodiesel, toxic properties have been adduced to the plant, especially the seeds. Thus, the current study was done with the aim of investigating the toxicity of the ethanol seed extract of *JC* in rats, mice and chicks; and also to use conventional antidotes to treat intoxication in rats due to *JC* poisoning.

The LD_{50} of the ethanol extract of the *JC* seed was determined by the method initially described by Lorke. In addition, acute behavioral and CNS toxicity studies of *JC* including antidotal therapy against *JC* poisoning were done. The data was analysed using SPSS and results were expressed as mean ± SEM. $p < 0.05$ was considered significant.

The LD_{50} of IP *JC* extract ranged from 177.48 to 288.53 mg/kg (moderately toxic) for the adult female rat, adult male mouse and young male rat. For the adult male rats the LD50 values were 565.69 mg/kg (IP, slightly toxic) and >5000 mg/kg (oral, slightly toxic) and the LD_{50} of the *JC* extract for the chicks was 28.28 mg/kg (IP, highly toxic). *JC* produced a fairly dose dependent behavioral and CNS depressant effects which were reduced by atropine, EDTA and a combination of atropine, sodium nitrite & sodium thiosulphate, and EDTA. Also these antidotes either singly or in combination reduced mortality among the rats by 25-50%.

In conclusion, the ethanol extract of *JC* seeds produces behavioral changes in experimental animals possibly in part by CNS depression which were ameliorated by atropine or EDTA and a combination of antidotes. Thus, these antidotes particularly atropine, may be exploited in the management of *JC* poisoning.

Key words: Acute toxicity, LD_{50}, antidotal, behavioral indices, *Jatropha curcas.*

***Corresponding Author: +232-78841262, dhmsamai@yahoo.com**

INTRODUCTION

Anecdotal evidence suggests that Sierra Leone has an ancient heritage of traditional medicine and a large proportion of the population depend on it for prevention and treatment of diseases. This is however not surprising as there is currently an increase interest and demand for herbal medicine (WHO AFRO, 2008). The World Health Organization over the years had encouraged, recommended and promoted traditional herbal remedies in national health care programmes as these drugs are readily available and affordable; and the people have faith in their curative abilities (WHO AFRO, 2008).

Jatropha curcas (physic nut or purging nut) is a drought resistant shrub or tree belonging to the family Euphorbiaceae, which is cultivated in Central and South America, South-East Asia, India and Africa (Martinez-Herrera, 2006). The plant contains alkaloids, lignans, cyclic peptides and terpenes such as diterpenes. Diterpenes from different species of *Jatropha curcas (JC)* have a wide range of biological activities including tumor-promoting, irritant, cytotoxic, anti-inflammatory, antitumor, molluscicidal, insecticidal and fungicidal activities (Rakshit *et al.*, 2010). In Africa and Asia, almost all parts of *JC* including seeds, leaves, bark and roots are widely used. For instance, the seeds as well as the oil are used as purgative, and in the treatment of scabies, gout, and dropsy (Neuwinger, 1994), skin diseases such as eczema, and to soothe rheumatic pain (Heller, 1996). During the past two decades, *JC* has attracted a lot of interest particularly for its oil, which can be used for biodiesel production (Kumar and Sharma, 2008).

In spite of the myriad of ethno medical uses and agro-feed potential of *JC* seeds and the potential for production of biodiesel, toxic properties have also been adduced to parts of the plant, especially the seeds (El Badawi *et al.*, 1995). Moreover, several cases of *JC* nut poisoning in humans have been reported following accidental consumption of the seeds with symptoms of giddiness, vomiting, diarrhoea and in extreme cases death (Abdu-Aguye, 1986).

The use of *JC* for biodiesel production may lead to the widespread cultivation of *JC* and this may increase the frequency of human or animal contact with the plant, seeds, or processed products. Thus, it is imperative to investigate and document its toxicity profile. The current study was therefore undertaken with the aim of investigating the toxicity of the ethanol seed extract of *JC* in rats, mice and chicks; and also to use conventional antidotes to treat intoxication in rats due to *JC* poisoning.

MATERIALS AND METHODS

Animals

Male and female Wistar rats (150-250 g), male Swiss albino mice (15-30 g) and Shika Brown chicks - cockerels and pullets (25-50 g) were used for this study. The animals were purchased from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University (ABU), Zaria, Nigeria except the chicks which were purchased from the National Animal Production Research Institute, Shika, also of ABU. The mice and rats were housed in a room kept at 23 – 27 ^{0}C and maintained on an approximate 12 h light / dark cycle; while the chicks were kept under continuous light exposure. Clean drinking water was provided *ad libitum* to all the animals. Mice and rats were fed with standard rodent feed prepared by Vital Feeds, Jos, Nigeria; while the chicks were fed with chick mash by Pfizer, Lagos, Nigeria. The experimental design and research plan together with animal handling and disposal procedures were approved by the Departmental Animal Ethical Committee ABU, Zaria. Ethical conditions of ABU, Zaria governing the conduct of experiment with experimental animals in line with international standards, were adhered to.

Plant Identification and Extraction Process

Dried *JC* fruits were collected in May 2012 at Calaba Town in the Eastern part of Freetown, Sierra Leone and were authenticated by a Taxonomist Alhaji B.M.S. Turay, Head, Department of Pharmacognosy and Dean, Faculty of Pharmaceutical Sciences, College of Medicine and Allied Health Science.

The *JC* dried fruits were de-husked to yield the seeds which were air-dried and pulverized using pestle and mortar. 1,035 g of the *JC* seed powder was weighed and defatted by Soxhlet extraction using 1.5 L petroleum ether, for 72h at room temperature. The marcs were dried and subjected to 70% ethanol filtration for 48h. The filtrates were evaporated and concentrated to dryness on a water bath set at 50°C. The ethanol extract obtained was placed in a desiccator to dry at room temperature and later stored in an airtight container for further use. The percentage yield of the plant was 4.56%.

Acute Toxicity Studies

Determination of Median Lethal Dose (LD_{50}) of the JC extract

The Median lethal dose (LD_{50}) of the ethanol extract of the *JC* seed was determined in two phases by the method initially described by Lorke with some modifications (Lorke, 1983). The rats and chicks were divided into three groups based on gender and age (i.e. adult male rats, adult female rats and young male rats; and 5 day old cockerel, 5 day old pullets and 2 day old cockerels); and all the animals received the extract intraperitoneally except for a subset of the male adult rats which was treated orally (**Table 1**).

Table 1: Design for LD_{50} determination and behavioral studies

Expt. No.	Species	Sex	Age	Weight (g)	Route	Factor	Phase I Doses $(mg/kg)^{+}$	Phase II Doses $(mg/kg)^{++}$
1	Rat	Male	Adult	110-140	i.p.	Standard	0, 10, 100, 1000	200, 400, 800, 1600
2	Rat	Male	Adult	110-140	p.o.	Route	0, 10, 100, 1000	800, 1600, 2900, 5000
3	Rat	Female	Adult	110-140	i.p.	Sex	0, 10, 100, 1000	140, 225, 370, 600
4	Rat	Male	Young	50-70	i.p.	Age	0, 10, 100, 1000	140, 225, 370, 600
5	Mouse	Male	Adult	17-22	i.p.	Species	0, 10, 100, 1000	140, 225, 370, 600
6	Chicks	Male (cockerel)	2-day	34-39	i.p.	Species / Age	0, 1, 10, 100	5, 10, 20, 40
7	Chicks	Male (cockerel)	5-day	39-48	i.p.	Species / Age	0, 1, 10, 100	5, 10, 20, 40
8	Chicks	Female (pullet)	5-day	34-48	i.p.	Species / Age / Sex	0, 1, 10, 100	5, 10, 20, 40

N=3 per group for phase 1 study and N= 1 per group for phase 2 study except for the chicks where N=4.

A total of 48 rats, 12 mice and 48 chicks were used for the phase 1 study. Briefly, pre-determined single IP doses of *JC* extract (0, 10, 100 and 1000 mg/kg) were administered to three animals per subgroup (adult male rat, adult female rat, young male rat, and adult male mice). An additional group of 12 adult male rats (3 per subgroup) was treated orally with similar doses of the *JC* extract. Three chick per subgroup (5-day old pullet, 5-day old cockerel and 2-day old cockerel) received pre-determined single IP doses of the *JC* extract (0, 1, 10 and 100 mg/kg). The number(s) of death(s) among the animals were recorded after 24h.

The doses of the extract used in the Phase 2 study were based on the outcome of the Phase 1 study

and are shown in **table 1**. A total of 16 rats, 4 mice and 48 chicks were used in this phase and the extract was administered to 1 rat or mouse per subgroup including the orally treated rat. However, four chicks per group received single IP doses of 5, 10, 20 and 40 mg/kg respectively. The number(s) of death(s) among the animals were also recorded after 24 hours.

The LD_{50} values was calculated as follows: $LD_{50} = \sqrt{}$ the Maximum Dose of Survival x $\sqrt{}$ the Minimum Dose of Death. Using the adult male rats as standard, factors such as gender, age, specie and route of drug administration which may affect the acute toxicity of the *JC* seed extract were evaluated using the computed LD_{50} data.

Acute Behavioral Toxicity of *JC* extract

During the phase 1 LD_{50} study, an equal number of a vehicular control group for the 5 day old cockerels, adult male rats and adult male mice, was treated intraperitoneally with normal saline (NS) for comparison of the behavioural indices with their respective *JC* extract treated group. The animals were observed for behavioral changes and signs of toxicity within a 2h period. The important behavioural indices and signs of toxicity as previously described by Fugner and Hoerks (Fugner and Hoerks, 1971) were recorded. In the absence of automated activity-recording cages the behavioural indices were assessed visually by trained Research Assistants. A total of 6 Research Assistants were trained and two observed and recorded behavioral changes for 4 animals. The means of their observations were computed. Except for movement and attempted escape the other behavioral indices were qualitatively graded as follows: 1 for mild, 2 for moderate and 3 for severe. The mean values were computed and compared to the control group.

Acute CNS toxicity of the *JC* extract

A total of 18 adult male Wistar rats (220-250 g) were used for this study. Under chloroform anaesthesia the head of each rat was shaved and the skin retracted, revealing the skull. The hyperstriatum (HS), optic tectum (OT) and pontine reticular formation (PRF) were implanted with stainless steel electroencephalogram (EEG) electrodes for EEG recordings. The semispinalis capitis muscle of the neck was similarly implanted with electromyogram (EMG) electrodes for EMG recordings. The electrodes were soldered to a plug and fixed over the skull with dental acrylic resin. The rats were allowed 24 hours recovery from the implantation before EEG-EMG recordings were done. Lead wires of the electrodes were connected to an EEG-EMG amplifier via a slip ring fixed above the cage allowing free movement of the rat within the cage. The amplifier was connected to the system for acquiring and processing the data. Each rat served as its own control with recording done 1h before and after drug treatment. Saline-treated controls were used to check that the instrument was functioning properly and that any detected response was not due to the procedure. The experimental rats (3 per group) were treated with a CNS stimulant amphetamine (20mg/kg), a CNS depressant chlorpromazine (50mg/kg) and ethanolic extract of *JC* seeds (0, 50, 100 and 200 mg/kg), intraperitoneally.

Antidotal therapy against acute *JC* extract toxicity in rats

Based on the anti-nutritional, heavy metal and toxic phytochemical constituents found in *JC* extract in our laboratory, and its folkloric use as a pesticide as well as the observation that respiratory failure was a constant feature of high doses during the LD_{50} study, antidotal therapy was designed against organophosphate, cyanide and heavy metal poisoning. Adult male Wistar rats used for this study were divided into six groups of 4 rats each based on weight. Rats in the first five groups were treated intraperitoneally with 10 mg/kg *JC* extract followed by IP injections of NS, atropine (20mg/kg), combination of sodium nitrite (25mg/kg) & sodium thiosulphate (1.25mg/kg), EDTA (40mg/kg), and a combination of all the antidotes respectively. Group six rats received IP NS twice, in place of the *JC* extract and the antidote.

The doses of the agents used were based on preliminary studies done with the extract, as well as previous experience with the antidotes in our laboratory. Trained Research Assistant familiar with the presentation of *JC* toxicity in rats, including respiratory distress, depression, sedation, stretching and rhythmic abdominal/thoracic muscle constriction, were used to record the signs of toxicity. The rats were observed for increased, decreased, or absence of toxicity signs 30 minutes after the administration of the antidotes, and thereafter every 30 minutes up to 2 hours. The number of deaths in each group after 24 was also recorded.

Statistical analyses and data presentation

The results were expressed as mean ± standard error of the mean (SEM) where appropriate. The data was analysed using Statistical Package for the Social Scientist (SPSS) software, version 14. Test of significance was done using one way analysis of variance (ANOVA) followed by Dunnett's Post-hoc tests for scaled variables and Kruskal-Wallis test for ranked variables. A value of $p < 0.05$ was considered significant.

RESULTS

Median Lethal Dose (LD50) of the JC extract

The LD_{50} of *JC* was determined for adult male and female rats, young male rats, adult male mice and chicks (5 day old pullet, 5 day old cockerel and 2 day old cockerel). Except for a subgroup of the adult male rats which received the *JC* extract orally, the animals in the other groups were treated intraperitoneally. Following the IP administration of the *JC* extract to the adult female rat, adult male mouse and young male rat the LD_{50} values ranged from 177.48 to 288.53 mg/kg with young male rat accounting for the least. The LD_{50} values of the *JC* extract following the IP and oral administration for adult male rats were 565.69 mg/kg and >5000 mg/kg respectively. On the other hand there was no variation in the LD_{50} value of the *JC* extract for the chicks (28.28 mg/kg) irrespective of their gender and age (**Table 2**).

Table 2: Determination of the LD50 values of *Jatropha curcas* in rats, mice and chicks

Species	Sex	Age	Weight (g)	Route	Factor	LD_{50} (mg/kg)	Classification
Rat	Male	Adult	112-142	i.p.	Standard	565.69	Slightly toxic
Rat	Male	Adult	112-142	p.o.	Route	>5000	Practically not toxic
Rat	Female	Adult	112-142	i.p.	Sex	288.53	Moderately toxic
Rat	Male	Young	50-70	i.p.	Species	177.48	Moderately toxic
Mouse	Male	Adult	17-22	i.p.	Species	288.53	Moderately toxic
Chick	Male (cockerel)	2-day	34-39	i.p.	Species/ Age	28.28	Highly toxic
Chick	Male (cockerel)	5-day	29-48	i.p.	Species/ Age	28.28	Highly toxic
Chick	Female (Pullet)	5-day	24-44	i.p.	Species/ Age/Sex	28.28	Highly toxic

The experimental animals were treated with pre-determined doses of JC extract intraperitoneally or orally in two phases for the determination of the LD_{50} of the extract. The dose of the extract used in phase was determined by the outcome of the phase 1 study. Following phases 1 & 2 treatments the number(s) of death(s) among the animals were recorded after 24 hours.

Acute Behavioral Toxicity of *JC* extract

The effect of the *JC* extract on behavioral changes and the production of toxic signs were assessed for 2 hours in the experimental animals. A vehicular control group similarly treated with NS was used for comparison of the behavioral indices. For the 5-day old cockerels no statistical significant different was noted in pecking and movement between the *JC* extract treated groups and their respective controls. The *JC* extract at the doses used produced a statistical significant increase in crouching, close eyes, sleep and respiratory distress when compared to the control groups (**$p<0.05$**) in a fairly dose dependent fashion. However, 10-100 mg/kg dose of the extract significantly reduced escape attempts when compared to their respective controls (**$p<0.05$; Table 3**).

With regards the adult male mice, the *JC* extract at the doses used produced statistical significant increase in stretching, inability to stand, sedation and respiratory distress ($p<0.05$); and statistically

significant decrease in feeding and movement (**p<0.05**) when compared to the respective controls (**Table 4**). However, with the exception of feeding which was significantly reduced in the adult male rats, at the doses used *JC* significantly increased stretching, inability to stand and respiratory distress (p<0.05), but produced no statistical significant effect on the movement of the rats when compared to their respective controls. In addition, a statistical significant increase in sedation by the adult male rats was produced by the highest dose of *JC* used (**p<0.05; Table 5**).

Table 3: The effects of ethanol extract of *JC* seeds on behavioral indices in 5-day old cockerels

Dose (mg/kg)	Pecking[1]	Movement[1]	Escape Attempts[1]	Crouching[1]	Close eyes[2]	Sleep[2]	Respiratory Distress[2]
0	2.24± 0.20	36.50±17.52	6.00±2.38	0.00±0.00	0.38±0.24	0.00 ±0.00	0.00±0.00
1	1.88± 0.20	33.17± 8.69	10.00±5.55	1.89±0.39*	1.87±0.22*	2.45±0.17*	1.67±0.22*
10	0.78±0.27	10.67± 4.76	0.00±0.00*	2.43±0.16*	2.37±0.26*	2.35±0.20*	1.44±0.35*
100	1.20± 0.48	27.17±13.47	0.00±0.00*	1.99 ±0.35*	2.34±0.22*	2.26±0.30*	1.65± 0.41*

*The cockerels were treated with JC extract (1-100 mg/kg) intraperitoneally and a vehicular control group was similarly treated with NS (0 mg/kg JC). They were observed for behavioral changes and signs of toxicity within a 2 hour period. Except for movement and attempted escape which were quantitatively assessed the other behavioral indices were qualitatively graded as follows: 0 –no effect, 1 for mild, 2 for moderate and 3 for severe. The mean values were computed and compared to the control group. N=3 per group; [1]Actual numbers counted; [2]Ranking from 0 (no effect) to 3 (maximum effect); Data are means ± SEM; * P<0.05 vs. control.*

Table 4: the effects of ethanol extract of *Jatropha curcas* seeds on behavioral indices in adult male mice

Dose (mg/kg)	Feeding[1]	Movement[1]	Stretching[1]	Inability to stand[1]	Sedation[2]	Respiratory distress[2]
0 (control)	12±2.74	11.00±4.63	0.00±0.00	5.33±2.76	5.17±2.79	0.17±0.17
10	0.00±0.00*	8.33±3.51	18.33±4.36*	14.00±5.15*	17.17±3.38*	10.50±3.13*
100	0.00±0.00*	3.33±1.28*	16.33±3.44*	16.00±5.81*	21.00±2.84*	10.83±2.95*
1,000	0.00±0.00*	6.50±1.26	16.50±4.20*	19.00±3.75*	21.50±2.59*	16.17±2.57*

*The adult male mice were treated with JC extract (10-1,000 mg/kg) intraperitoneally and a vehicular control group was similarly treated with NS (0 mg/kg JC). They were observed for behavioral changes and signs of toxicity within a 2 hour period. Sedation and respiratory distress were qualitatively graded as follows: 0 –no effect, 1 for mild, 2 for moderate and 3 for severe. The mean values were computed and compared to the control group. N=3 per group; 1Actual numbers counted; 2Ranking from 0 (no effect) to 3 (maximum effect); Data are means ± SEM; * P<0.05 vs. control*

Dose (mg/kg)	Feeding[1]	Movement[1]	Stretching[1]	Inability to stand[1]	Sedation	Respiratory distress

0	7.33±2.93	9.0±3.84	0.33±0.33	7.5±4.97	9.17±3.89	1.50±1.50
10	0.83±0.40*	18.67±8.74	19.67±3.05*	11.0±2.85*	12.83±3.46	9.00±3.03*
100	0.33±0.33*	24.67±5.46	25.75±2.65*	12.33±2.03*	9.50±3.03	14.17±3.39*
1,000	0.33±0.33*	26.17±9.42	19.00±2.98*	17.33±2.82*	17.17±3.91*	22.33±2.67*

Table 5: The effects of ethanol extract of *Jatropha curcas* seeds on behavioral indices in adult male rats

*The adult male rats were treated with JC extract (10-1,000 mg/kg) intraperitoneally and a vehicular control group was similarly treated with NS (0 mg/kg JC). They were observed for behavioral changes and signs of toxicity within a 2 hour period. Sedation and respiratory distress were qualitatively graded as follows: 0 –no effect, 1 for mild, 2 for moderate and 3 for severe. The mean values were computed and compared to the control group. N=3 per group; [1]Actual numbers counted; [2]Ranking from 0 (no effect) to 3 (maximum effect); Data are means ± SEM; * P<0.05 vs. control.*

Acute CNS toxicity of the *JC* extract

In order to investigate the CNS effects of the *JC* extract EEG tracings of rats were separately compared with that produced by a conventional CNS stimulant and CNS depressant. Thus, adult male rats were treated intraperitoneally with amphetamine (20 mg/kg), or chlorpromazine (50 mg/kg) or varying does of *JC* (0, 50, 100 and 200 mg/kg). As expected amphetamine (20 mg/kg) and chlorpromazine (50 mg/kg) produced EEG tracings consistent of CNS stimulation and depression respectively. The CNS effect produced by *JC* was dose-dependent and similar to that of chlorpromazine (**Figure 1&2**).

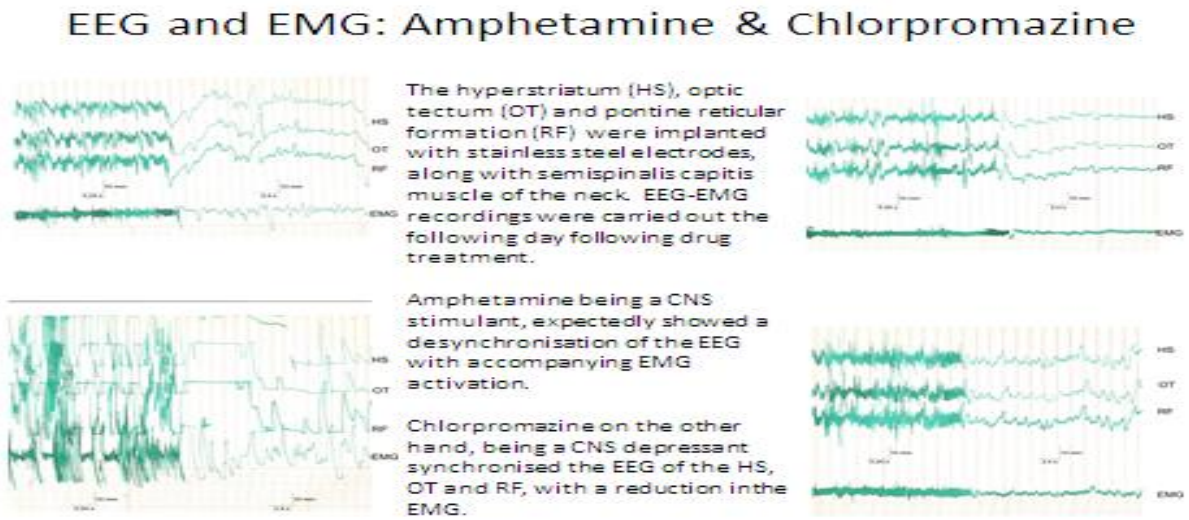

Figure 1*: the effect of amphetamine (20 mg/kg) and chlorpromazine (50 mg/kg) on EEG and EMG recordings in rats. The rats were treated with single doses IP amphetamine (left) or chlorpromazine (right) and EEG-EMG recordings were done 24 hour later.*

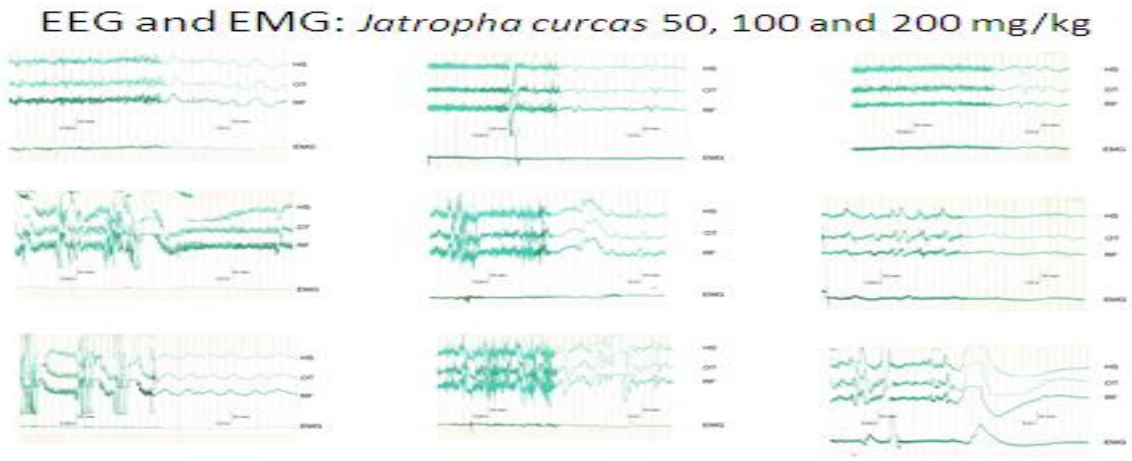

Figure 2: the effect of varying doses JC extract (50-200 mg/kg) on EEG and EMG recordings in rats. The rats were treated with single doses of 50 mg/kg JC (left), 100 mg/kg JC (center) and 200 mg/kg JC (right) and 24 hours later the EEG-EMG recordings were done.

Reduction of the toxic effect of the *JC* extract by conventional antidotes

The conventional antidotes at the doses used had no statistically significant effect on the *JC* extract toxicity up to 60 minutes (**results not shown**). At 90 and 120 minutes atropine and the combination antidotes (i.e. atropine, sodium nitrite & sodium thiosulphate, and EDTA) at the doses used significantly reduced the *JC* toxicity (**p<0.05; Figures 3A & 3B**). In addition, 40 mg/kg EDTA significantly reduced *JC* intoxication at 120 min (**p<0.05; Figure 3B**). Mortality among the rats was reduced by 50% with atropine (20 mg/kg) whilst sodium nitrite 25 mg/kg & sodium thiosulphate1.25 mg/kg, EDTA 40 mg/kg, and combination antidotes each reduced mortality by 25%.

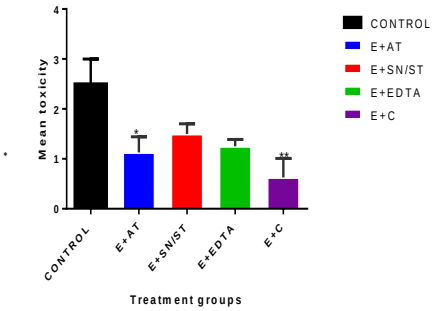

Figure 3A

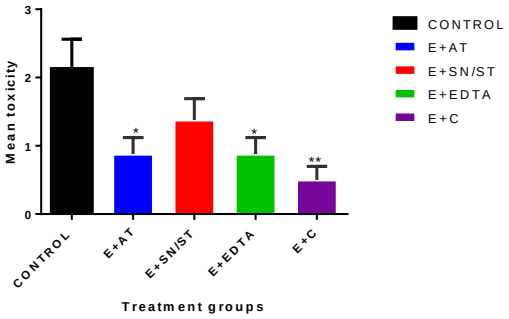

Figure 3B

Figure 3(A-B) *the effect of conventional antidotes {atropine (AT), Sodium nitrite and sodium thiosulphate (SN/ST), ethylene diamine tetra acetate (EDTA), and combination of three groups (C)} in reducing the toxic effect of JC extract (10 mg/kg). Rats were divided into five groups and were treated intrperitoneally with JC (E) only, E and Atropine (E+AT), E and Sodium Nitrite & Sodium thiosulphate (E + SN/ST), E and EDTA (E + EDTA) and E and combination of the three antidotes (E + C) respectively. The mean toxicity produced by JC (E) was recorded represented in the figure as control, and the ability of the antidotes to reduce the toxic effect of JC within (A) 30 minutes, (B) 60 minutes, (C) 90 minutes and (D) 120 minutes was noted. Data are means of triplicate observations ± SEM with n=4 per group.*P<0.05 vs. control group; **P<0.01 vs control.*

DISCUSSION

The result of the current study shows that the LD_{50} of the IP ethanol extract of *JC* for the adult female rat, adult male mouse and young male rat range from 177.48 to 288.53 mg/kg with young male rat accounting for the least; where as the LD50 values of the *JC* extract following the IP and oral administration for adult male rats were 565.69 mg/kg and >5000 mg/kg respectively. In addition, the LD50 value of the *JC* extract for the chicks was 28.28 mg/kg irrespective of their gender and age. Thus the LD_{50} values of the ethanol extract of *JC* seed showed specie, gender and age variation. According to the classification by Matsumura (1975) and Corbett and Colleagues (1984) the ethanol seed extract of the JC is moderately toxic to the adult female and young male rats and adult male mice, slightly toxic to the adult male rats, highly toxic to the chicks (Matsumura, 1975; Corbett *et al*.; 1984). These latter authors independently classified agents with LD_{50} values of less than 1, 1-50, 50-500, 500-5000, 5000-15000 mg/kg and greater than 15 g/kg as being extremely toxic, highly toxic, moderately toxic, slightly toxic, practically non-toxic and harmless respectively.

Unlike IP administration of the *JC* extract, oral administration produced practically no toxic effect to the adult male rats further demonstrating route variation. The gender and age variation in the *JC* toxicity seen in rats may account in part for the exclusion of females and young children from most therapeutic clinical trials. That no gender or age differential was noted in the acute toxicity of the extract in chicks as a specie was not surprising given that the 2-days and 5-days are so close, and below what one may term 'pubertal' when sex differences normally become evident. With respect to specie difference, chicks were more susceptible to the effects of the extract as evidenced by the least LD_{50} followed by mice and rats. The specie variation in toxicity may be relevant given that *JC* is sometimes used as a household fence, and unintentional poisoning could occur in domestic animals as well as humans. The highest susceptibility of chicks to the *JC* seed extract toxicity demonstrated in the current study is in consonance with an earlier study by El-Badawi and colleagues in 1995 in which they reported high incidence of mortality among Brown Hisex chicks fed with diets containing 0.5% *JC* seed (El-Badawi *et al.;* 1995).

Unlike the current study which reports slight toxicity of the ethanol extract of IP *JC* seeds in rats, the methanol extract of IP *JC* seeds from Nigeria was reported by Oluwole and Bolarinwa in 1997 to be highly toxic in rats with an LD_{50} value of 25.19 mg/kg (Oluwole and Bolarinwa, 1997). Furthermore, the methanol extract of *JC* stem bark and leaves from India was reported to be slightly toxic in rats following IP and oral administration with an LD_{50} of 2,000 mg/kg (Sacdeva *et al.;* 2012) and 2,500 mg/kg (Mishra *et al.;* 2012) respectively. These variations may not be unrelated to the differences in the toxic materials of *JC* from different parts of the world.

In the current study, the ethanol extract of *JC* seeds in the 5-day old cockerels produced behavioral changes such as significant increase in crouching, closing of the eyes, sleep and respiratory distress and a significant decrease in escape attempts; with decrease in food intake and uncoordinated movement. With regards the adult male mice and rats, the *JC* extract at the doses used produced significant increase in stretching, inability to stand, sedation and respiratory distress, and significant decrease in feeding. Although movement in the adult male mice was significantly reduced by the *JC* extract, it was not altered in the adult male rats. The EEG recordings in the adult male rats following the administration of amphetamine produced desynchronisation of the EEG with accompanying EMG activation consistent of CNS stimulation. Chlorpromazine on the other hand, being a CNS depressant produced a synchronized EEG pattern of the hyper striatum, optic tectum and reticular formation, with a reduction in the EMG; which is also consistent with CNS depression. The EEG recordings of the ethanol extract of the *JC* seeds showed pattern that was very similar to chlorpromazine; a CNS depressant effect which was dose dependent.

Thus the behavioral effects were fairly dose dependent and could be due to loss in skeletal muscle tone or CNS depression. The CNS depressant effects of several species of the *Jatropha* plant have been alluded to by several researchers

including Apurba and colleagues (2013), Akanmu and colleagues (2005), Wannang and colleagues (2004), and Fojas and colleagues (1986). These researchers have earlier reported EEG recordings consistent of CNS depression by extracts of *JC* leaves (Fojas *et al.;* 1986), *Jatropha gossypifolia* fruits (Apurba *et al.;* 2013), *JC* seeds (Wannang *et al.;* 2004) and *Stachtarpheta cavennesis* (Akanmu *et al.;* 2005) in rodents. Generally it is presumed that behavioral changes result from changes in the number of activated dopamine receptors. Although dopamine had been showed to be an inhibitory neurotransmitter in the brain which could produce behavioral changes (Bloom *et al*.; 1975), it had been documented to have both inhibitory and excitatory actions in different parts of the brain (Meller *et al*.; 1989, Wambebe and Osuide, 1980). Thus, the behavioral changes and CNS depressant effects produced by *JC* in this study may not be unrelated to its effects on dopamine either directly or indirectly by altering the balance between dopaminergic and cholinergic activities. The latter effect is further supported by the fact that the toxic effect of the *JC* extract in rats was ameliorated by atropine.

For the antidotal therapy studies, the signs of toxicity (decreases in food intake, movement and escape attempts; plus increases in stretching, sedation and respiratory distress) of the ethanol extract of *JC* seed at 30 and 60 min were not reduced by none of the antidotes at the doses used. However, at 90 and 120 minutes atropine and the combination antidotes (i.e. atropine, sodium nitrite & sodium thiosulphate, and EDTA) at the doses used significantly reduced the *JC* extract toxicity; whilst 40 mg/kg EDTA significantly reduced *JC* intoxication at 120 min. Mortality among the rats was reduced by 50% with atropine whilst EDTA, the combination of sodium nitrite and sodium thiosulphate, and the combination of all the antidotes, each reduced mortality by 25%.

The reduction in the toxicity produced by the ethanol extract of the *JC* seeds by atropine indicates that *JC* may possess either a direct or indirect cholinergic activity. As the ethanol extract of *JC* seeds have been shown in our laboratory to contain heavy metal it is highly probable that EDTA may have reduced the toxicity by *JC* by chelation of heavy metals. Additionally, *JC* was shown in our laboratory to contain cyanide and most of the rats which presented with signs consistent of cyanide toxicity died. Although the combination sodium nitrite-sodium thiosulphate did not significantly diminish signs of *JC* toxicity it reduced mortality among the rats by 25% suggestive of cyanide involvement.

In conclusion, the ethanol extract of the *JC* seeds produces behavioral changes in experimental animals possibly in part by CNS depression and these effects are ameliorated by atropine or EDTA and a combination of antidotes. Thus, these antidotes particularly atropine, may be exploited in the management of Jatropha poisoning in animals and humans.

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