

**EVALUATION OF THE TOXICOLOGICAL PROFILES OF
JOBELYN[®]**

BY

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SUMMARY

JOBELYN® is widely distributed and used in Nigeria. There is limited report on its toxicological profile. This study is carried out to examine the acute and short-term chronic toxicity profiles of this herbal formulation. The main objectives are to determine the LD₅₀, gross morphological effects and histopathological effects.

Albino mice, mean weight 16.45 ± 3.14g, were used in this study. Acute studies, involving the administration of graded concentrations of JOBELYN® both orally and intraperitoneally as single doses, revealed that the formula produces some toxic effects at sufficiently high dosage. The LD₅₀ values following oral and intraperitoneal routes of administration were 215.06 mg/kg (r = 0.916) and 193.37 mg/kg (r = 0.995) respectively. The major behavioral/morphological effects were reduction in motor activity, piloerection and sedation, particularly at the high doses. The sub-lethal doses did not significantly modify the normal behavioral repertoire of licking, grooming and sniffing. Histopathological examination also did not indicate severe pathological changes. At the lethal doses, some degree of congestion was noticed in the lung, liver splenic and kidney tissues. Short-term chronic studies, involving the intraperitoneal administration of sub-lethal doses daily for 14 days showed that the formula did not produce further toxic effects. Initially, mild sedation and piloerection were noticed but these soon disappeared. Histopathological examination revealed only mild congestion in the organs. None of the animals died during the period of evaluation of sub-chronic toxicity. The LD₅₀ value and the tolerance limits recorded would indicate a profound safety profile for this product. At the current dosage regimens recommended by the manufacturers, one can infer from these studies that the possibilities of exhibiting toxicities in the clinics are very remote. Further toxicological evaluation in rats is being conducted.

INTRODUCTION.

There is a worldwide recognition of the vital role of herbal medicines, as a tool in traditional medicines, in healthcare (WHO, 1996). As much as 80% of people in developing world are said to depend on traditional medicines for primary healthcare (Bodekar, 1994). The economic situation of such people may be responsible for this large-scale dependence on traditional medicines. To this end, herbal medicines have been shown over the years to have intrinsic utility, which should be promoted, and its potentials developed for wider use and benefits of mankind (Hans 1989). Infact, the Alma Ata declaration of 1978 encouraged the use of all available resources for primary healthcare and recommended that government should give high priority to using traditional health practices and incorporate proven traditional remedies into National Drug Policy and Regulations (Zhang, 1994). Unfortunately, most of the herbal medicines are poorly regulated and controlled. There is a dearth of scientific proofs of effectiveness and safety of herbal preparations, which is required for marketing authorization.

JOBELYN®, manufactured by Health Forever Products Ltd., Lagos, Nigeria, is a commercial herbal preparation that is widely distributed and used. It is claimed to stimulate rapid production of red blood cells and maintains the integrity of white blood cells even with the presence of viral or bacterial infections. JOBELYN also strengthens the immune system and thereby enhances body's defensive mechanisms.

Unpublished reports indicate its usefulness in the management of anemic states. The manufacturers recommend it as remedy for anemia in Sick cell anemia, Cancer, HIV/AIDS, Malaria, Typhoid fever, Aplastic anemia and Pregnancy.

However, the clinical efficacy of an herbal product is not enough to recommend its use as medicines. Its safety and tolerability must also be ascertained.

Preliminary Pilot Preclinical studies in mice showed that JOBELYN® does not create an abnormality in blood parameters. Further, it was shown that serum creatinine and cholesterol levels were significantly lowered. The studies did not reveal significant adverse effects (Okochi et al, 1999a). In

addition, unpublished studies revealed the antitrypanosomal activities of JOBELYN® (Okochi et al, 1999b). Unpublished toxicological evaluation of JOBELYN® in mice also showed that the product has lethal effects at high doses and is more toxic than Sorghum bicolor (L) (Dada, 2000).

The clinical use of the drug to date has not also revealed any significant adverse events.

This report details the toxicological profile of JOBELYN® in mice. The main objective is to evaluate its toxicity profiles after acute and short-term chronic administration.

Composition of JOBELYN®

JOBELYN® is claimed to be a unique formulation produced from tropical plants.

It is a dark brown powder with characteristic taste and odor.

It is basically a glycoprotein derived from tropical plants. Laboratory analysis shows the presence of carbohydrates, protein, tannins, saponins, coumarins and iron. It does not contain any alkaloid. The herbal ingredients is Sorghum bicolor.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS.

Healthy Albino mice -males and females; average weight, 16.45 – 3.14 g; weight range 13.75g – 19.48g were obtained from the Animal House of the College of Medicine, University of Lagos. The animals were kept in clean cages (10 mice/cage) in well-ventilated room and allowed unrestricted access to livestock feeds (from Ladokun feeds, Ibadan) and fresh water. They were also allowed to acclimatize to the environment for one week before each experiment. During this period of acclimatization, the animals were periodically assessed for gross morphological/behavioral changes. The animal cages were cleaned out of waste alternate days.

EXPERIMENTAL DESIGNS

Acute Toxicity Testing:

To determine dose-response effects, sub-lethal and lethal doses, and to calculate the LD₅₀

Oral Route.

42 mice were divided into 7 equal groups (A – G). Mice in groups A to F were administered 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml and 0.7ml of 20% solution of JOBELYN® oral. The animals in group G that served as control, were given 0.3ml of deionized water orally. The animals were monitored for gross morphological and behavioral changes over 72hrs. The LD₅₀ was determined using probit analysis within 95% confidence limit. The animals were sacrificed and essential organs subjected to histological examination. The organs were preserved in 10% formaldehyde solution for 4 weeks before processing.

Intra-Peritoneal Route.

36 mice were also divided into 6 equal groups (A – F). Mice in groups A to E were given 0.2ml, 0.3ml, 0.4ml, 0.5ml and 1.0ml of the stock solution of JOBELYN® intraperitoneally whilst animals in group F, serving as control, received 0.3ml of deionized water IP. The animals were monitored for gross behavioral changes over 72 hrs. The LD₅₀ was determined using probit analysis within 95% confidence limit. The animals were sacrificed and essential organs subjected to histological examination. The organs were preserved in 10% formaldehyde solution for 4 weeks before processing.

Sub-acute Toxicity Testing: short-term chronic toxicity

30 mice were divided into three equal groups (A – C). Mice in groups A and B were given 0.1ml and 0.2ml of 20% solution of JOBELYN® IP daily for 2 weeks, whilst the last group, C, serving as control were given 0.3ml deionized water IP daily for 2 weeks. The animals were monitored for morbidity and mortality. They were sacrificed after 15 days and essential organs subjected to histological examination. The organs were preserved in 10% formaldehyde solution for 4 weeks before processing.

MONITORING PARAMETERS.

Two major parameters were used:

1. Gross Morphological & Behavioral Effects. This included the assessment for changes in locomotor activity, piloerection, normal behavioral repertoire (grooming/licking/biting), sedation, aggressiveness, catalepsy, appetite, urination, defecation, vomiting, sneezing/wheezing.
2. Histopathological Effects. This included the evaluation for pathological changes.

HISTOPATHOLOGICAL PROCESSING.

The various organs were processed using the automatic tissue processor. The technique involves dehydrating the well-fixed 3mm-sized tissues placed in tissue baskets with their respective labels by passing them through graded alcohol. They were then moved into Xylene solution baths and then placed in molten wax for impregnation. The solidified blocks were trimmed and sectioned using the Rotary microtome at 5µ thickness. Sections were then floated on water bath at 50°C and picked up using albuminized microscopic slides. The cut sections were dried on hot plates at 60°C and then stained by haematoxylin and Eosin (H & E) to demonstrate tissue structures.

Stock Solution of JOBELYN®

20g of JOBELYN® was soaked in 100mls of deionised water and kept refrigerated over 48hrs. The solution was then filtered and used as stock/test solution. Fresh stock was prepared every 4 days and the solution always kept refrigerated when not in use.

The weight of JOBELYN® in 2ml of the stock solution was determined by comparing the weight of 2mls of deionized water with 2mls of JOBELYN® stock solution. The concentration of 20% w/v JOBELYN® stock solution was determined to be 8.2mg/ml.

RESULTS

ACUTE TOXICITY TESTING

ORAL:

0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, and 0.7ml of stock solution was administered orally. The summary of the observed changes in behavior is recorded in table 1. The mortality rates and probit analysis report are recorded in tables 2 a & b respectively. In summary, reduced mobility accompanied the administration of JOBELYN® from the 0.3ml dose level within 1hr. However, the animals were alert, except at high doses where a slight degree of sedation was noted. There was also some degree of piloerection and the surviving animals recovered motility soon after 48hrs. The normal behavioral repertoire was maintained; no aggressiveness. There was no noticeable reduction in appetite or changes in urine output. There was, however, some difficulty encountered with the oral administration of the product, as the animals were rather small in size.

Table 1. Observed Behavioral Changes (acute Oral administration)

Dose (ml)	Observations.
Control	Normal behavioral repertoire; no sedation; no piloerection over the 72hrs of observation.
0.1ml	Mobile, alert, grooming and licking; slight piloerection. Very active over the 72hrs of observation
0.2ml	Slight reduction in motility; normal repertoire, slight piloerection. Active over the 72hrs of observation. No mouse died.
0.3ml	Reduced mobility, slight sedation and piloerection over 24hrs. Recovered motility, active and feed normally over the next 48hrs. 1 mouse died after 24hrs.
0.4ml	Reduced motility, sedation and piloerection over 24hrs. 2 mice died after 24hrs. Recovered motility after 36hrs although not very active. Fully recovered after 48hrs
0.5ml	Marked reduction in motility, sedation and piloerection over 48hrs. 3 mice died within 24hrs. Recovered motility after 72hrs
0.7ml	Marked reduction in motility. 5 mice died within 24hrs.

Table 2a. Mortality Rates after acute Oral administration

Dose(mls)	Dose (mg)	Dose (mg/kg)	# mice	# mice that died	% Mortality.
Control: 0.0	0.0	0.0	6	0	0
0.1	0.82	49.85	6	0	0
0.2	1.64	99.70	6	0	0
0.3	2.46	149.54	6	1	17
0.4	3.28	199.39	6	2	33
0.5	4.10	249.24	6	3	50
0.7	5.74	348.95	6	5	83

Table 2b. Probit Analysis Result (acute oral administration)

Dose (mg)	mortality	% Mortality	Probit +5
0.00	0/6	0	0.0
0.82	0/6	0	0.0
1.64	0/6	0	0.0
2.46	1/6	17	4.0458
3.28	2/6	33	4.5601
4.10	3/6	50	
5.74	5/6	83	5.9542

LD₅₀ value = 215.06 mg/kg

r = 0.916

Confidence limit = (147.30 – 313.99) mg/kg

INTRA-PERITONEAL.

0.2ml, 0.3ml, 0.4ml, 0.5ml and 1ml of the stock solution were administered intraperitoneally to different groups of 6 mice. The summary of observed

behavioral changes, mortality rates and probit analysis are presented in tables 3 and 4 respectively.

Table 3. Observed Behavioral Changes after acute IP administration

Dose (ml)	Observations
Control 0.0	Normal behavioral repertoire. Alert.
0.2	Normal behavioral repertoire. Slight piloerection. 1 mouse died after 24 hrs. Full recovery after 48hrs
0.3	Slight reduction in motility, mild sedation over 24hrs. 2 mice died within 24hrs. Recovers fully after 48hrs
0.4	Reduced motility, sedation, piloerection over 48 hrs. 3 mice died after 24 hrs.
0.5	Reduced motility, marked sedation over 48hrs. 4 mice died after 24 hrs
1.0	Reduced motility, marked sedation over 12hrs. All died within 24hrs

Table 4a. Mortality Rates after acute IP administration

Dose (ml)	Dose (mg)	Dose (mg/kg)	# mice	# mice that died	% Mortality
Control 0.0	0.0	0.0	6	0	0
0.2	1.64	99.70	6	1	17
0.3	2.46	149.54	6	2	33
0.4	3.28	199.39	6	3	50
0.5	4.10	249.24	6	4	67
1.0	8.20	498.48	6	6	100

Table 4b. Probit Analysis Result (acute IP administration)

Dose (mg)	Mortality	% Mortality	Probit +5
0.0	0/6	0	0.0
1.64	1/6	17	4.0458
2.46	2/6	33	4.5601
3.28	3/6	50	
4.10	4/6	67	5.4399
8.20	6/6	100	

LD₅₀ value = 193.37 mg/kg

r = 0.995

Confidence limit = (131.54 – 284.25) mg/kg

SHORT-TERM CHRONIC TOXICITY TESTING.

0.1ml and 0.2ml of the stock solution were administered to 2 groups of ten mice each daily for 2 weeks. The third group received 0.2ml of deionized water daily for 2 weeks and served as control.

The observed behavioral changes are presented in table 5.

Table 5. Observed Behavioral Changes after subchronic IP administration.

Dose (ml)	Observations
Control	Normal behavioral repertoire throughout the 14 days of observation. No mice died.
0.1	Normal behavioral repertoire. Slight piloerection. No mice died. Slight reduction in appetite
0.2	Reduced motility, piloerection. Slight sedation. No mice died. Slight reduction in appetite.

PATHOLOGICAL REPORT.

Histopathology: acute and subchronic studies.

Hearts: No marked pathological changes revealed
Lungs: Moderate congestion noticed at lethal doses
Liver: Moderate congestion noticed at lethal doses
Kidneys: No marked pathological changes revealed
Spleen: Slight congestion noticed in lethal doses.

DISCUSSION

Albino mice are commonly utilized in the evaluation of toxicity profiles of various chemical agents. They were utilized in this study for this purpose and also because they are readily available.

Acute toxicity studies revealed that JOBELYN® would produce lethal consequences in sufficiently high dosage. The LD₅₀ values for Oral and Intraperitoneal routes were found to be 215.06 mg/kg and 193.37 mg/kg respectively. The respective confidence limits are 147.30 – 313.99 mg/kg and 131.54 – 284.25 mg/kg

The manufacturers recommend as much as 500mg per dose (2 capsules) and 1.5g per day (6 capsules). For any average adult weighing 70kg, this translates to a dosage of about 7.14mg/kg per dose and 21.42 mg/kg/day. Even though the extrapolation of data from animals to humans is anticipated and not definitive, the recommended dosage regimen in man can be said to be comparatively very safe. For the oral route, the product has a tolerance limit of 99.70mg/kg. This gives a large room for dosage manipulation, which may be applicable to man.

The major behavioral changes were Reduced Motility and Sedation.

These effects may not be mutually exclusive; i.e. one could be responsible for the other. These effects may also have been responsible for the seeming loss of appetite that was observed at high doses in this study. An earlier unpublished report indicated inactivity, loss of appetite, dehydration and impaired vision induced by JOBELYN®. In this study, the oral route caused some spillage of the test substance on the eyes, which precipitated some degree of eye irritation. This was not noticed with the Intraperitoneal route. Dehydration was not observed in this study. It should also be noted that the surviving animals recovered full motility soon after 48hrs after administration.

The histopathological report revealed no significant pathological changes in the organs studied. There was however a moderate degree of congestion in the liver and lungs, and focal tubular necrosis in the kidneys particularly at the high, lethal doses.

CONCLUSION.

These studies have shown that JOBELYN® in very large doses have toxic properties. However, the histopathological report has not revealed any significant and alarming changes in the specie of animals used in this study. The LD₅₀ value and the tolerance limits recorded would indicate a profound safety profile for this product. At the current dosage regimens recommended by the manufacturers, one can infer from these studies that the possibilities of exhibiting toxicities in the clinics are very remote. As far as adverse drug reactions are concerned, the corresponding effects in man to the observed reduced motility and sedation in mice need further investigation during clinical trials. These may be suggestive of interference with biogenic amines (neurotransmitters) especially Dopamine and Noradrenaline or simply a non-specific depression of the CNS in mice. Similarly, the clinical component of tissue/organ congestion at lethal doses will need to be looked into during clinical trials. It can be deduced from these studies that JOBELYN® has a good safety profile, and is wholesome and safe for human use, as recommended by the manufacturers. Further toxicological screening in rats is being conducted.

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