IOSR Journal Of Pharmacy www.iosrphr.org
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 8, Issue 4 Version. III (April 2018), PP. 09-14

Microbial Evaluation And Subchronic Toxicity Studies Of Ade & Ade Herbal Drug Formulated For Systemic Detoxification And Diuretic Agent.

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ABSTRACT
OBJECTIVE: This study Evaluated Microbial Purity and Sub chronic toxicity studies of Ade and Ade Herbal Drug (AHD) in Wister's rats.
MATERIALS AND METHODS: Microbial purity was evaluated using appropriate diagnostic media. Acute toxicity of the product was evaluated in Swiss albino mice. In Sub chronic toxicity studies, Swiss albino rats weighing 98g-174g were divided into four study groups (n=5 in each cage) and fed with different treatment doses of the formulation for 30days as indicated below:
Control group received 0.2ml saline solution
Group l received 600mg/kg extract
Group ll received 1,200mg/kg extract
Group lll received 1,800mg/kg extract
At the end of the 30days, the albino rats were sacrificed and blood samples were collected into EDTA and Heparin Bottles respectively for Haematological and Biochemical analyses using standard kits.
RESULTS - The LD_{50} of the product showed no lethality at the highest dose (5,000mg/kg) employed and contained microbial load. Significant ($P<0.05$) reduction in the RBC values at a dose (600mg/kg and 1,200mg/kg) and significant ($P<0.05$) increase at a dose (1,800mg/kg) compared with the control groups with reduction in the MCH and MCHC values indicates poor blood formation. There were significant reductions in the TC, HDL- cholesterol, LDL – cholesterol and TG values in all the treated groups compared with the control groups. Therefore, the formulation is beneficiary to cardiovascular risk factors. Significant changes in the weight of the Lung, Heart Liver and kidney but non significant ($P>0.05$) changes in the values of pancreas compared with the control groups and non significant increase in the AST values in all the doses (600-1,800mg/kg) but significant ($P<0.05$) reductions in the ALT values. There was gained in the weight in all the treated rats throughout the study.
CONCLUSION- The study revealed that the formulation contained high microbial contaminant which were above acceptable level and would be considered microbiologically unsafe for human consumption. While its sub -chronic toxicity study showed that the formulation reduces cholesterol level at low and high doses in animals, it may be beneficial on cardiovascular risk factor. Consequently, the formulation had no side effect on the liver, heart and the kidney in all the doses, however, the changes observed in all the doses on different physiological parameters, suggests phytochemical screening of the formulation and adherence to Good Manufacturing Procedures(GMPs)

Key words- Ade and Ade herbal drug, Microbial purity, acute toxicity and Sub chronic toxicity

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Date of Submission: 14-04-2018 Date of acceptance: 30-04-2018

I. INTRODUCTION

Plants and herbs derived medicine are popularly known as herbal medicine and are generally regarded as safe based on their long standing use in various cultures (Mosihuzzaman and Iqbal Choudhary, 2008). Herbal medicines remain the main stay of health care system in the developing countries and are gaining increasing popularity in the developed countries where orthodox medicines are predominantly used. The herbal medicine has the advantage of being effective as well as being a cheap source of medical care (Ogbonnia et al, 2010a). Also, there is growing disillusion with modern medicine coupled with the misconception that herbal products being natural could be assumed of being devoid of adverse and toxic effects associated with conventional and

allopathic medicines. Herbal medicines could be contaminated with microbial and foreign materials such as heavy metals, pesticide residues or even aflatoxins resulting from the unhygienic manner by which they were prepared.

The presence of any of the possible contaminants may lead to a potential health risk to the vast population that depends on these preparations for their health care needs, increased morbidity and mortality associated with the use of herbs has raised universal attention in the last few years (Bandaranyake, 2006). Upon exposure, the clinical toxicity may vary from mild to severe and even life threatening, making the safety and toxicity evaluations of these preparations imperative. Herbal medicine is most often poly herbal, being prepared from mixtures of different plant parts obtained from various plant species and families and may contain multiple bioactive constituents that could be difficult to characterize (Ogbonnia et al, 2010b). The bioactive principles in most herbal preparations are not always known and there could be possibilities of interaction with each other in solution. The quality as well as the safety criteria for herbal drugs may be based, therefore, on a clear scientific definition of the raw materials used for such preparations. Also herbal medicine may have multiple Physiological activities and could be used in the treatment of varieties of disease conditions (Pieme et al, 2006).It could be administered in most disease states over a long period of time without proper dosage monitoring and consideration of toxic effects that might arise from such prolonged usage(Ogbonnia et al, 2010c). The danger associated with the potential toxicity of herbal therapies employed over a long period of time demands that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs (Tedong et al, 2007, Ogbonnia et al., 2008). ADE and ADE herbal formulation is one of such poly herbal formulations used as systemic detoxifier and diuretic agent. However, sub chronic toxicity evaluation is required to establish potential adverse effects of this highly valuable poly herbal formulation that is now widely consumed for its physiological benefits.

The aim of this study is to evaluate the safety of the formulation of ADE and ADE by investigating its microbial purity and sub chronic toxicities animals to establish potential adverse effects of this formulation.

II. MATERIALS AND METHODS

Test material

Ade and Ade herbal drug was supplied by Traditional Birth Attendant (TBA) in powder formulation. The therapeutic label claimed that the formulation was prepared with unspecified quantities of the listed plants and the prescribed daily recommended dose for human adult ½ teaspoon daily and children aged 5 to 12 years ¼ teaspoon daily. The drug is used for systemic detoxifiers and as diuretic agent. The production date and batch number of the herbal drug were also not indicated. 35g of Ade and Ade herbal drug was dissolved in 500ml and gave a stock concentration of 70mg/ml. The drug extract was kept in the laboratory refrigerator until the time of drug administration. Upon attainment of room temperature $(28 \pm 2)^{oc}$, appropriate volumes of the preparation were administered directly to the experimental animals via a stainless steel cannula.

Animal

Adult albino rats (98 – 174)g and Swiss albino mice (40±2)g of either sex were used for sub chronic profiling and acute toxicity studies. The animals were supplied by the Animal Facility Centre of the College of Medicine, Lagos University Teaching Hospital, Idi Araba Lagos, Nigeria. The animals were fed with grower mash and had free access to water and maintained under standard conditions of humidity, temperature and 12/12 ratio light and darkness cycle. The animals were allowed to acclimatised before the commencement of the study and standard protocol was drawn up in accordance with the Good Laboratory Practice(GLP) regulation of WHO(1998). The principles of laboratory animal care were also followed in this study.

Microbial purity

Determination of micro - organisms was done on the product using WHO 2011 quality control methods for herbal materials.

Acute toxicity studies

The acute toxicity (LD_{50}) was estimated p.o. in 36 mice of nine group (n=4 per group in each cage) following Lorke's method (1983). The dose levels used ranged from 100 to 5,000mg/kg and numbers of death per group in each cage within 72hours administration of the drug was noted.

Sub chronic toxicity studies

Male and female rats were selected randomly and divided into four experimental groups of five rats each of both sexes. After fasting the animals overnight, the first group served as control received 0.2ml saline solution daily and the remaining three groups were given 600mg/kg, 1,200mg/kg and 1,800mg/kg of AHD orally for 30 days. At the first day (Day 0) initial weight of the animals were recorded following the

administration of the drug extract. The animals were weighed on sensitive balance every five days from the start of the treatment to note any body weight variation. At the end of the experiment, they were made unconscious under chloroform anaesthesia and blood collected via cardiac puncture in two tubes: one EDTA for immediate analysis of haematological parameters, the second with heparin to separate plasma for biochemical estimation. The liver, heart, lung, pancreas and kidney were dissected out. Washed in saline solution and weighed (Ogbonna et al, 2010). The collected blood was centrifuged within 20mins of collection at 4000rpm for 10 min to obtain the plasma, which was analysed for total cholesterol, total glyceride and HDL – cholesterol levels by precipitation and modified enzymatic procedures from Sigma Diagnostics (Wasan et al., 2001). LDL – cholesterol levels were calculated using Friedwald equation (Crook, 2006).Plasma was analysed for alanine amino tranferase (ALT), aspartate aminotransferase(AST), and Creatinine by standard methods(Sushura etal, 2006), Hematocrit was estimated using the method of (Ekaidem et al.,2006). Haemoglobin contents were determined using Cyanmethaemoglobin (Drabkin) method (Ekaidem et al,2006).

Statistical analysis

The results are expressed as means ± standard error of the mean (SEM) were analysed as a complete randomised design using one way analysis of variance (ANOVA)

III. RESULTS

Table1: Showed massive growth (TNTC) for bacterial organisms in Casein Soya-bean Digest Agar media within 24hours at 37^{o}C culture. There were presence of Salmonella spp, Pseudomonas spp and Shigella spp at 37^{o}C in 24 hours incubation with Xylose Lysine Deoxycholate Agar, Triple Sugar Iron Agar and Deoxycholate citrate Agar, and in Mac Conkey agar. While for the presence of Clostridium spp, was determined by Cooked Meat Medium (WHO 2011)

Table 1: Microbial purity test

MEDIA	E. coli	Salmonella spp.	Pseudomonas spp.	Shigella spp.	Clostridium spp.	BTVC	FTVC
CSDA						TNTC	
SDA							nil
MAC	Nil			+ve			
DCA							
XLD		+ve		+ve			
TSI		+ve		+ve			
CA			+ve				
CMM					+ve		

CA – Cetrimide Agar, CMM – Cooked Meat Medium, CSDA – Casine Soya-bean Digest Agar, DCA - Deoxycholate Citrate Agar, MAC – Mac Conkey Agar, SAD – Sabouraud Dextrose Agar, TSI – Triple Sugar Iron Agar, XLD – Xylose Lysine Deoxycholate Agar, BTVC-Bacterial total viable count and FTVC- Fungal total viable count

Acute toxicity studies

The acute toxicity study shows that there was no lethality arises from the mice that received different doses of AHD, also, there were no changes in their feeding habits, stool, urine and they were active throughout the 72H observational study.

Weekly body weight

TABLE 2: From Day 0 – Day 30, there were changes in the body weight of all the rats in the treated groups throughout the study. Rats treated with doses 600mg/kg and 1,800mg/kg AHD gained body weight in a similar fashion with the control groups. However, rats treated with 1,200mg/kg had non significant reduction in the body weight from Day 20 – Day 30 when compared with the control groups

Table 2; Mean body weight changes in rat treated with various doses (600 – 1,800mg/kg) of AHD for 30 days against the control

Treatment	**DAY 0**	**DAY 5**	**DAY10**	**DAY 15**	**DAY 20**	**DAY 25**	**DAY 30**
Control	98±6.9	120±9.0	138±10.4	147±11.4	155±15.0	158±15.0	167±14.8
600mg/kg	103±8.1	185±17	193±19.6	185±18.2	177±20.2	177±22,6	189±21.8
1200mg/kg	174±17	154±15	156±13.7	147±17.4	153±11.8	152±12.7	161±21.1
1800mg/kg	124±14.7	161±23	166±21.1	170±21.1	156±16.9	161±17.9	167±20.0

n=5.value(Mean ±SEM), *P≤0.05significant (one way anova) versus control

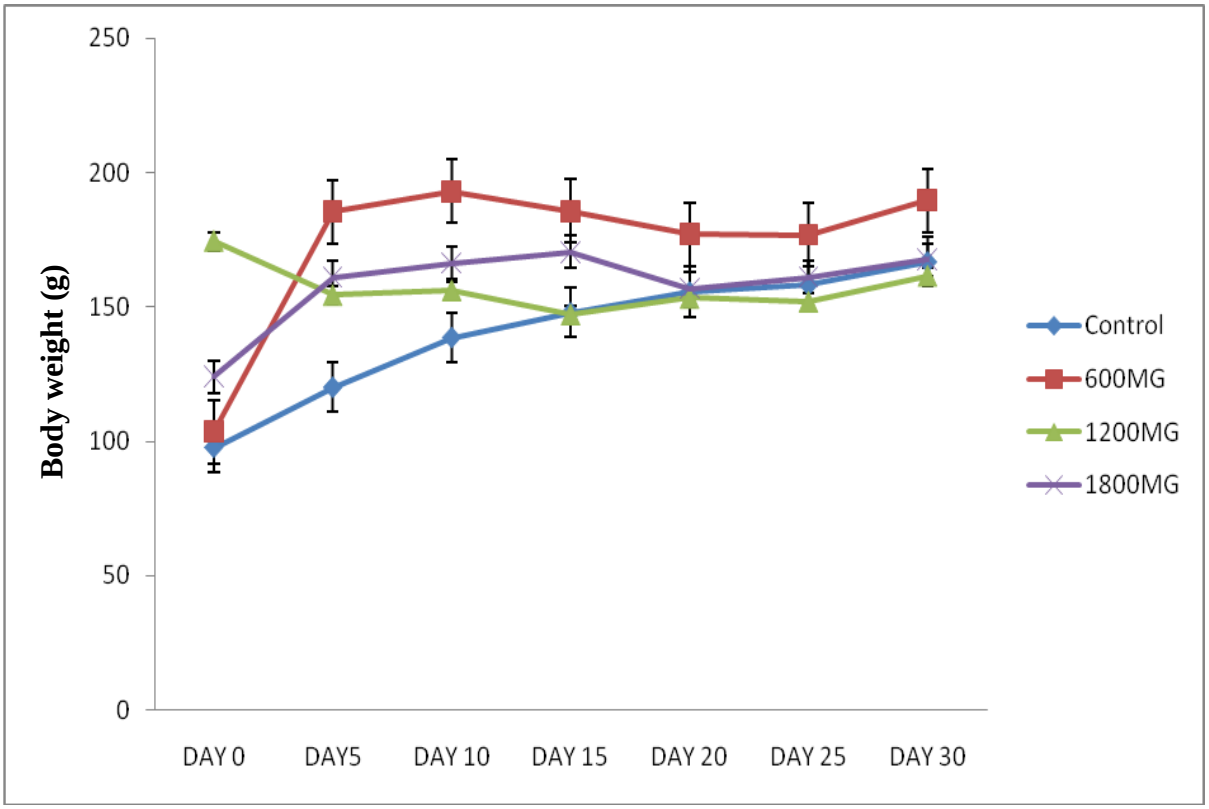

Fig2: Mean body weight changes in rat treated with various doses (600 – 1,800mg/kg) of AHD for 30 days against the control n=5.value (Mean ±SEM), *P≤0.05significant (one way anova) versus control

Organ weight

In table 3, there were significant (P<0.05) changes in the weight of Lung, Heart, Liver and Kidney in the rats treated with doses 600mg/kg – 1,800mg/kg AHD compared with the control groups. However, changes observed in the weight of pancreas compared with the control group were not significant.

Table 3: Mean organ weight changes in rat treated with various doses (600 -1,800mg/kg) of AHD for 30 days against the control

Treatment	Lung	Heart	Pancreas	Liver	kidney
Control	1.18±0.06	0.50±0.04	0.30±0.03	5.40±0.60	0.48±0.58
600mg/kg	1.08±0.1*	0.68±0.04*	0.50±0.06	7.00±0.63*	0.48±0.12*
1200mg/kg	1.12±0.07*	0.48±0.04*	0.26±0.04	4.80±0.48*	0.44±0.74*
1800mg/kg	1.82±1.3*	0.56±0.05*	0.72±0.32	6.30±0.35*	1.26±0.29*

n=5.value (Mean ±SEM), *P≤0.05significant (one way anova) versus control

Biochemical profile

Table 4: There were significant (P<0.05) reduction in TC, HDL, LDL values in the rats treated with doses 600mg/kg – 1,800mg/kg AHD compared with the control groups. Non significant reduction in the triglyceride (TG), total protein (TP) and alanine amino transfarease (ALT) values compared with the control group were observed. Slight increase in the aspartate aminotransferase(AST) and CREATININE values compared with control groups at doses 1,200mg/kg and 1,800mg/kg but the increase were non significant.

Table 4: Mean Biochemical profile in rat treated with various doses (600 – 1,800mg/kg) of AHD for 30 days against the control

Treatment	Control	600mg/kg	1,200mg/kg	1,800mg/kg
AST(u/l)	33.20±1.59	33.40±2.6	43.80±2.6	37.20±4.1
ALT(g/dl)	30.40±1.03	26.40±1.5	28.20.±1.7	2.40±2.4
TC(mmol/l)	164±10.8	110.±7.8*	129±8.4*	99±4.3*
HDL(mmol/l)	31.80±3.18	21.4±2.5*	22.8±1.96*	19.00±1.58*
LDL(mmol/l)	124±10.6	82.00±8.8*	100±10.5*	74.6±4.7*
TG(mmol/l)	40.0±2.9	32.20±1.9	33.4±3.8	29.6±1.6

TP(g/dl)	6.16±0.16	5.92±0.06	5.66±0.19	5.7±0.11
CREA(mg/dl)	0.82±0.03	0.68±0.37	0.74±0.24	0.92±0.37

n=5.value (Mean ±SEM), *P≤0.05significant (one way anova) versus control
Aspartate aminotransferase(AST),Alanine aminotransferase(ALT),total cholesterol(TC),high density lipoprotein(HDL),low density lipoprotein(LDL),triglyceride(TG), total protein(TP) and Creatinine

Haematological profile

In table 5, there were significant in the RBC values in all the rats treated with the doses 600mg/kg – 1,800mg/kg AHD compared with the control groups. Also, changes in the values of WBC, HGB, HCT, MCV, MCH, MCHC, PLT and PCT. Though, the changes observed in these values were not significant compared with the control groups

Table 5: Mean Haematological profile in rat treated with various doses (600 – 1,800mg/kg) of AHD for 30 days against the control

TREATMENT	**Control**	**600mg/kg**	**1,200mg/kg**	**1,800mg/kg**
WBC(10^3/I)	12.7±2.84	11.3±1.26	56.6±30.5	8.15±0.69
RBC(10^6/I)	5.95±0.38	5.44±0.32*	3.92±1.03*	6.23±0.10*
HGB(g/dl)	11.0±0.68	10.2±0.55	10.2±0.36	11.9±0.05
HCT (%)	33.7±2.37	31.2±1.88	26.2±5.68	35.3±0.05
MCV(fl)	56.6±0.94	57.4±0.87	64.1±5.63	56.7±0.90
MCH(pg)	18.5±0.37	18.8±0.23	70.9±34.7	15.9±1.60
MCHC(g/dl)	32.8±0.32	32.8±0.68	93.3±40	33.7±0.19
PLT(10^9)	570±116	688±59.3	845±259	628±165
PCT (%)	0.42±0.20	0.52±0.11	0.42±0.24	0.18±0.04

n=5 value (Mean ±SEM), *P≤0.05significant (one way anova) versus control
WBC- White Blood Cell, RBC- Red Blood Cell, HGB- Haemoglobin, HCT- Haematocrit,
MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Haemoglobin, MCHC-Mean Corpuscular Haemoglobin Concentration, PLT-Platelet and PCT- Procalcitonin

IV. DISCUSSIONS

The microbial purity evaluation of the formulation in table 1 showed the presence of Salmonella spp, Pseudomonas spp, Shigella spp and Clostridium spp in various diagnostic media also massive growth (TNTC) of Total Aerobic Bacteria above the acceptable limits. The fungi and E. coli were found below the World Health Organization official microbial limit. The presence of the pathogens may be attributed to poor hygiene, contaminated water and failure to abide by the principles and procedure of Good Manufacture Practice (GMP)

Herbal medicine have received greater attention as alternative to clinical therapy in recent times leading to subsequent increase in their demands (Sushruta et al, 2006). In rural communities, exclusive use of herbal medicine, prepared and dispensed by herbalists without formal training for the management of various ailments is still a very common practice requiring that experimental screening method be established to ascertain the safety and efficacy of these herbal products. AHD in powder dosage form is one of such remedies prepared as a mixture from different plant species as herbal medicine used locally for diuretic and systemic detoxification.

From the result in fig 1, there were gained in body weight of all the treated rats with doses 600mg/kg and 1,800mg/kg AHD throughout the study. But slight non significant reduction in the body weight of rats treated with dose 1,200mg/kg from Day 20 – Day 30 compared with the control groups. The changes may be attributed to the environmental factors. In a similar fashion, there were significant $P<0.05$ changes in the weight of the lung, heart, liver and kidney but non significant changes value of pancreas when compared with the control groups. Non significant $P>0.05$ increase in AST values in all the treated rats compared with the control groups indicate that the formulation may not have any adverse effects on the cardiovascular functions. Also non significant $P>0.05$ reduction in the ALT values in all treated rats compared with the control groups shows that the formulation has no deleterious effects on the liver functions.

In fig 3, there were significant $P<0.05$ reduction in the TC,HDL,LDL and TG values in all the treated rats compared with the control groups indicates that the formulation had hypo lipidaemia effects, which had beneficial effects on cardiovascular risk factors. Non significant reduction in the creatinine values at doses 600 and 1,200mg/kg in the treated rats respectively and non significant increase in the value of creatinine at a dose 1,800mgkg compared with the control groups couple with the decrease in the values of TP in all the treated groups compared with the control suggests that the formulation had no deleterious effects on the renal functions

As it is presented in fig4, there were significant $P<0.05$ reduction in the RBC values at doses 600mg/kg and 1,200mg/kg of the formulation in the treated group also significant increase in the treated groups at a dose

1,800mg/kg compared with the control groups. Non significant decrease in all the treated groups compared with the control showed poor absorption of iron. Changes in the values of WBC in all the treated groups were observed. But at dose 1,200mg/kg the was non significant increase in the value of WBC compared with the control groups .also, increase in the PLT values in all the treated groups compared with the control group this increase in the number of platelets suggests infection which might arises from the unhygienic environment or contaminants that might be present in the formulation.

V. CONCLUSION

The study revealed that the formulation contained high microbial contaminant which were above acceptable level and would be considered microbiologically unsafe for human consumption. While its sub - chronic toxicity study showed that the formulation reduces cholesterol level at low and high doses in animals, it may be beneficial on cardiovascular risk factor. Consequently, the formulation had no side effect on the liver, heart and the kidney at low and high doses, however, the changes observed at low and high doses on different physiological parameters, suggests phytochemical screening of the formulation and adherence to Good Manufacturing Procedures (GMPs)

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Oguntoyinbo Abel "Microbial Evaluation And Subchronic Toxicity Studies Of Ade & Ade Herbal Drug Formulated For Systemic Detoxification And Diuretic Agent." IOSR Journal of Pharmacy (IOSRPHR), vol. 8, no. 04, 2018, pp. 09-14