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Original Research Article

Microbial, Phytochemical Screening and Toxicity Analysis of GM PLUS Herbal Drug

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Abstract

This study was design to examine the microbial load, phytochemical screenings and acute toxicity of GM Plus herbal drug mixture. Six samples of the mixture were sent to the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana for analysis. For the toxicity test, Sprague-Dawley rats were put into six rats in each group. Animals in all groups were immunized with $5.0*10^8$ SRBC/ml after which each group was treated with either GM Plus (30,100 or 300 mg/kg p.o., daily) based on preliminary investigations, levamisole (10mg/kg, p.o, daily), dexamethasone sodium (4mg/kg, i.m, daily) or Normal saline. The result of the toxicity proved that, Levamisole and 30,100, and 300 mg/kg GM Plus treatment resulted in significant increases ($p<0.001$) in the HA titre and WBC count relative to the dexamethasone and no treatment groups. A differential count performed indicated an increase in neutrophil proportion of the total count in the GM Plus treated groups. With regards to the microbial analysis, the total microbial load of Gm Plus was within the acceptable limits (BP 2015; category C of herbal products) there were no pathogenic microorganisms present. The herbal mixture has complied with the BP specifications for microbiological quality. The product GM Plus is therefore safe for usage.

Keywords: GM Plus, microbial load, toxicity, herbal drug, animal model.

INTRODUCTION

The Product GM Plus is an herbal mixture formulated for use in humans. It contains the following ingredients; Allium sativum, rosemarinus officinalis, Eugenia caryophyllata, citrus limonin. The World Health Organization advocates the use of herbal medicines that are proven scientifically to be of good quality, safe and efficacious as affordable alternatives to orthodox medicines for the majority of the world's populace particularly in low-income countries [1] this is an essential requirement for the registration of newly developed medicinal products [2- 4]. The study was done as requested by the Food and Drugs Authority (FDA) of Ghana as part of the registration requirements of the herbal product.

METHODOLOGY

Department of Pharmaceutics

Three tests were conducted at the Kwame Nkrumah University of Science and Technology, KNUST, are discussed here from three different departments.

Table-1: Microbial quality analysis

Test	Results	Specification (BP 2015)
Total Aerobic Microbial Count (TAMC)	$1.0*10^4$cfu/ml	$<5.0*10^5$cfu/ml
Total Yeast/Moulds Count (TYMC)	$1.0*10^3$cfu/ml	$<5.0*10^4$cfu/ml
Bile-tolerant Gram-negatives (Enterobacteria)	3.5*10cfu/ml	$<1.0*10^4$cfu/ml
Eschericha col (MaC;37^{o}c; 48h)	Not detected	Absent (in 1ml)
Salmonella (BSA; 37^{0}C;48h)	Not detected	Absent (in 25ml)

REMARKS

The total microbial load of Gm Plus was within the acceptable limits (BP 2015; category C of herbal products) there were no pathogenic microorganisms present. The herbal mixture has complied with the BP specifications for microbiological quality.

Department of Herbal Medicine

Table-2: Phytochemical and Physicochemical studies test conducted

PHYTOCHEMICAL AND PHYSICOCHEMICAL STUDIES	
GM PLUS	
NAME	GM PLUS
INDICATION	Immune Stimulant
ACTIVE INGREDIENTS	Allium sativum, rosemarinus officinalis, Eugenia caryophyllata, citrus limonin
DATE OF MANUFACTURE	17/11/2016
DATE OF EXPIRY	17/11/2018
BATCH NUMBER	Not stated
PRODUCED BY	Livelong Herbal Centre, P.O Box AC 402, Art Centre, Ashongman Estate, Accra

1.ORGANOLEPTIC PROPERTIES	
Form	Liquid
Colour	Light yellow
Taste	Characteristic
Odour	Characteristic
2.PHYSICOCHEMICAL PROPERTIES	
Ph	3.54
Dry weight per mL	0.1884
Specific gravity/ mL	1.0209
3.PHYTOCHEMICAL PROPERTIES	
Reducing sugars	Positive
Saponins	Negative
Alkaloids	Negative
Flavonoids	Positive
Phytosterols	Negative
Terpenoids	Positive
Tannins	Positive
4**. TLC CHROMATOGRAPHIC PROFILE**	
Stationary phase	- Pre-coated silica gel plates
Mobile Phase	Chloroform: Pet-ther (9:1)
Sample used	Chloroform extract
Detecting reagent	Anisaldehyde
Results	Five (5) prominent spots were deserved after spraying and gently warming. Three (3) yellow spots (Rfs;0.90, 0.77, 0.25 Two (2) pink spots (Rfs; 0.47, 034)

DEPARTMENT OF PHARMACOLOGY

TEST CONDUCTED: Efficacy, Acute and Sub-Chronic Toxicity Test

Sprague-Dawley rats were put into six rats in each group. Animals in all groups were immunized with $5.0*10^8$ SRBC/ml after which each group was treated with either GM Plus (30,100 or 300 mg/kg p.o., daily) based on preliminary investigations, levamisole (10mg/kg, p.o, daily), dexamethasone sodium (4mg/kg, i.m, daily) or Normal saline.

Hemagglutinating antibody titer

On day 20, the hemagglutinating antibody (HA) titer was determined. Blood samples were collected from retro-orbital plexus into test tubes placed on ice. After 1 h, the blood samples were centrifuged to obtain the serum. Ten serial dilutions of the serum in 0.15m phosphate buffer saline (PBS) pH 7.2 were made and 50ul of these were then titrated with 25ul of $2.5x10^7$ SRBC/ml. the test tubes were then incubated at room temperature for 2 h and examined visually for agglutination. The reciprocal of the highest dilution of serum showing 50% agglutination is expressed as HA titer. On the day 28, blood samples obtained from the rats were analyzed for the white cell profile using the CELL-DYN 1800 auto analyzer. The mean of the results obtained was recorded for each treatment group.

RESULTS

Levamisole and 30,100, and 300 mg/kg GM Plus treatment resulted in significant increases ($p<0.001$) in the HA titre and WBC count relative to the dexamethasone and no treatment groups. A differential count performed indicated an increase in neutrophil proportion of the total count in the GM Plus treated groups.

Toxicity Test

Table-3: Toxicity test on GM Plus

Species And strain	No. of animals Sex/group	Route of admin	Formulations and Dosage	Time of deaths and periods of observation	NOAEL	Symptoms
Sprague-Dawley Rats	25 males 5 groups (N=5	Oral	Freeze-dried sample 10,30,100,30,1000mg/kg	No death occurred during the period of observation: 24 hours, and 30days	>300mg/kg	Nil

NOAEL: No-Observable-Adverse-Effect Level

REMARKS

GM Plus exhibits immunostimulatory activity. The no-observed-adverse-effect level for was less than 300mg/kg following oral administration in Sprague-DAWLEY RATS.

CONCLUSION

Per all the tests conducted, the product is safe in experimental animals.

ACKNOWLEDGMENT

To the investigators at the KNUST who conducted the tests on the herbal product.

REFERENCES

1. WHO. (2000). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine. Geneva, Switzerland
2. Nyarko, A.K., Asiedu-Gyekye, I.J., & Sittie, A.A. (2005). A manual of Harmonised Procedures for Assessing the safety, efficacy and quality of plant medicines in Ghana. Ghana National Drugs Program, Ministry of Health. Yamens press ltd, Accra, 1-2
3. Robinson, S., Chapman, K., Hudson, S., Sparrow, S., Spencer-Briggs, D., Danks, A., & Bruce, C. (2009). Guidance on dose level selection for regulatory general toxicology studies for pharmaceuticals. London: NC3Rs/LASA.
4. Parasuraman, S. (2011). Toxicological screening. Journal of pharmacology & pharmacotherapeutics, 2(2), 74.