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EVALUATION OF PHARMACOLOGICAL PROFILE OF KOJU®- A NIGERIAN POLYHERBAL FORMULATION, IN WISTAR RATS

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ABSTRACT

Polyherbal formulations are mixtures of many plant parts obtained from various plant species and families. These plant combinations usually contain a wide array of bioactive compounds making them suitable for the treatment and management of a variety of disease conditions. These formulations are prepared most often and dispensed by individuals without formal training. As a result, experimental screening method is important in order to ascertain the efficacy and safety of these herbal products. This study investigated the pharmacological profile of Koju® (KPF) - a common Nigerian polyherbal formulation developed from *Xylopia aethiopica* (root bark), *Securidaca longepedunculata* (stem bark), *Magnifera indica* (stem bark), *Allium sativum* (seed), *Citrus aurantifolia* (root bark), *Morinda lucida* (seed) and *Saccharum officinarum*. The pharmacological profile was evaluated via investigation of its anti-hyperglycaemic potential (using alloxan- induced diabetes rat model), anti- bacterial potential (using *in vitro* action against bacterial isolates), anti- malarial potential (using *P. berghei berghei* inoculated mice model), analgesic potentials (using hot plate method), anti- inflammatory potential (using egg albumin- induced paw oedema model) and anti- pyretic (using bakers' yeast induced hyperpyrexia model). Results showed that KPF reduced hyperglycaemia in rats, Inhibited *salmonella spp* isolate *in vitro* and *P. berghei berghei* in mice and exhibited analgesic and inflammatory effect in rats. It was concluded that anti-hyperglycaemic, anti- bacterial, anti- malarial, analgesic and anti- inflammatory activities are part of the pharmacological profile of Koju polyherbal formulation.

KEYWORDS: Pharmacological, Koju®, Polyherbal, Formulation.

INTRODUCTION

Global use of herbs/ herbal products has increased tremendously over the past three decades.[1] More than 80% of people in Africa and Asia use herbal medicines and an increasing number in the Western world.[2] This extensive use could be attributed to the advantages of being efficacious and also a cheap source of medical care. There is also a growing disillusion with modern medicine coupled with the misconception that herbal products being natural may be devoid of adverse and toxic effects associated with convectional and allopathic medicines.[3] As a result, a large and increasing number of patients use medicinal herbs or seek the advice of medical personnel regarding their use. This interest has led to increasing scientific scrutiny of the therapeutic potentials of medicinal herbs with the aim of providing data to help patients and clients make wise and informed decisions about their use.

Polyherbal formulations are mixtures of many plant parts (which could be roots, leaves, stem, flowers, pods and seeds) obtained from various plant species and families. These plants/ their combinations usually contain an array of bioactive compounds making them suitable for the treatment and management of a variety of disease conditions.[4] It is generally believed that polyherbal formulations are just effective as the conventional drugs or more potent against diseases when taken alongside conventional drugs. By using herbal combinations, nature provides a balance of ingredients that may act as buffers, synergists or counterbalances, which work in harmony to rid the body of diseases and infirmities.[5] Some polyherbal extracts have been scientifically proven for efficacy in the treatment of diseases while many others are yet to be investigated.[6] One of such polyherbal formulations that is yet to be scientifically investigated for efficacy is Koju®.

Koju® is a polyherbal formulation consisting of *Xylopia aethiopica* (root bark), *Securidaca longepedunculata* (stem bark), *Magnifera indica* (stem bark), *Allium sativum* (seed), *Citrus aurantifolia* (root bark), *Morinda lucida* (seed) and *Saccharum officinarum* (Bark). It is usually indicated for a wide range of diseases which include menstrual disorders, pile, diorrhoea, dysentery, waist pain, protruding rectum, diabetes e.t.c. It is also claimed to possess aphrodisiac properties. The pharmacological profile of each component of the polyherbal formulation is highlighted below:

Xylopia aethiopica commonly called Negro pepper, African pepper, Guinea pepper and spice tree, is an ever green aromatic tree growing up to 15-30 m high. It is a native to the low land

rain forests and moist fringe forests in the savanna zones and coastal regions of Africa. The plant has been used as abortifacients, ecbolics as well as in the treatment of diarrhoea, dysentery; stomach disorder, menstrual disorder, naso-pharyngeal infections, arthritis, rheumatism, infections, among others.[7]

Securidaca longepedunculata is a small tree up to 6 meters high with a pale grey, smooth bark and oblong, more or less hairless alternate leaves that are variable in size and shape and crowded towards the stem tips.[8] Different parts of the plant has been reported to be useful in the treatment of venereal diseases, syphilis, pains, fever, epilepsy, pneumonia, tuberculosis, dislocated jaw, headaches, skin cancer, skin infections, and contraceptive purposes.[9], [10],[11],[12]

Magnifera indica is a juicy stone fruit which belongs to the family of Anacardiaceae in the order of Sapindales and is grown in many parts of the world, particularly in tropical countries. Various parts of the plants are used as astringent, acrid, refrigerant, styptic, anti-syphilitic, vulnerary, anti-emetic, anti-inflammatory and constipating. They are useful in vitiated conditions of pitta, metrorrhagia, calonorrhagia, pneumorrhagia, lecorrhoea, syphilis, uteritis, wounds, ulcers and vomiting. The juice of fresh bark has a marked action on mucous membranes, in menorrhoea, leucorrhoca, bleeding piles and diarrhoea.[13]

Allium sativum can rightfully be called one of nature's wonderful plants with healing power. It can inhibit and kill bacteria, fungi, lower (blood pressure, blood cholesterol and blood sugar), prevent blood clotting, and contains anti-tumor properties. It boosts the immune.[14] It has the ability to stimulate the lymphatic system and it is also considered an effective antioxidant. It prevents some forms of cancer, heart disease, strokes and viral infections. The sulfur containing compounds found in garlic afford the human body with protection by stimulating the production of certain beneficial enzymes.[15]

Citrus aurantifolia is native to tropical Asia but it is also found in all tropical and subtropical country. It is easily available plant showing a wide range of uses in treatment of various diseases. It is used traditionally as laxative, appetizer, stomachic, digestive, anthelmintic, dyspepsia, flatulence and helmenthiasis.[16] It is also used for cold fevers, sore throats, sinusitis and bronchitis, as well as helping asthma. Its oil is mainly used as antidepressant because it promoted refreshment to the tide mind. It can be helpful for rheumatism arthritis,

obesity and has an astringent and toning action to clear oily skin and acne, in the treatment of herpes, cuts and insect bites.[17], [18]

Morinda lucida (Rubiaceae) is a tropical West Africa rainforest tree also called Brimstone tree. In Cote d'Ivoire, it is locally called Sangogo or Bondoukou alongua while in Ghana, it is known as Twi, Kòn kròmà or Ewe amake while among the Yoruba natives (South-West Nigeria), it is called Òruwó.[19] Different parts of the plant are used for the treatment of fever, abdominal colic and intestinal worm infestation, as astringent for dysentery; it is trypanocidal and also has antimalarial activities and aortic vasorelaxant effect.[20], [21], [22], [23]

Saccharum officinarum commonly known as Sugarcane, Noblecane is widely cultivated throughout tropic and subtropic regions. It is used as a folk medicine and also used as an antidote, antiseptic, antivenomous, bactericide, cardio- tonic, demulcent, diuretic, intoxicant, laxative, pectoral, refrigerant, and stomachic. It is a folk remedy for arthritis, bedsores, boils, cancer, colds, cough, diarrhoea, dysentery, eyes, fever, hiccups, inflammation, laryngitis, opacity, skin, sores, sore throat, spleen, tumors, and wounds.[24]

The aim of this study was to evaluate the pharmacological profile of Koju, a polyherbal formulation that is widely consumed in Nigeria for its health benefits by investigating its anti-hyperglycaemic, anti- bacterial, anti- malarial, analgesic, anti- inflammatory and anti- pyretic potentials (which are some of the pharmacological properties that the marketers of this formulation claim that it possesses).

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Test Substance

Koju® is a polyherbal formulation developed by Kojumaribi Trado Medical Nig. Ltd. It contains *Xylopia aethiopica* (root bark) (3.5%), *Securidaca longepedunculata* (stem bark) (5.5%), *Magnifera indica* (stem bark) (7.5%), *Allium sativum* (seed) (8.5%), *Citrus aurantifolia* (root bark) (9.5%), *Morinda lucida* (seed) (10.5%), *Saccharum officinarum* (Bark) (25.0%) and water (q. s).

2.1.2 Drugs, Chemicals and Test Organisms

Metformin hydrochloride, Aspirin, Chloroquine (Health Seal Pharmacy Ltd., Lokoja, Alloxan monohydrate (Sigma- Aldrich) *E. coli*, *Salmonella* species and *Staphylococcus* species

(clinical isolates obtained from the Microbiology Diagnostic Laboratory of Ahmadu Bello University Veterinary Teaching Hospital, Zaria, Chloroquine- sensitive Plasmodium berghei berghei (P. berghei berghei) (NK65), Brilliant Green Agar (BGA), MacConkey agar, Bakers' yeast, Egg albumin.

2.1.3 Equipments

Electric hot plate, thermometer, FineTest® Glucometer (With corresponding test strips), Animal cages.

2.1.4 Experimental animals

Wistar rats weighing 180–250g and Albino mice weighing 16- 25g were procured from the animal house facility of the Department of Biochemistry, Salem University, Lokoja, and handled according to the guide for the Care and Use of Laboratory Animals, published by the National Institute of Health (NIH), USA. The rats were maintained at 25.0 ± 2 ^{O}C on a 12 h light/dark cycle with access to standard animal feed and water ad libitum for 7 d before the commencement of the experiment.

2.2 Methods

2.2.1 Preparation of Koju®

The plant materials- *Xylopia aethiopica* (root bark), *Securidaca longepedunculata* (stem bark), *Magnifera indica* (stem bark), *Allium sativum* (seed), *Citrus aurantifolia* (root bark), *Morinda lucida* (seed) and *Saccharum officinarum* (Bark) were bought from 'Old Market' in Lokoja, kogi State, Nigeria and authenticated by an Ethno-botanist at the Herbarium unit of the Department of Biological Sciences, Federal University Lokoja. The samples were thoroughly washed, thereafter dried and pulverized, using electric blender. The crude powders obtained from the plants materials were mixed in appropriate proportions [(according to the product label), *Xylopia aethiopica* root bark (175g), *Securidaca longepedunculata* stem bark (275g), *Magnifera indica* stem bark (375g), *Allium sativum* seed (425g), *Citrus aurantifolia* root bark (475g), *Morinda lucida* seed (525g), and *Saccharum officinarum* Stem bark (1250g)] was weighed and extracted with 10000 ml (10L) of distilled water. After 48 hours, the mixture was filtered using muslin sieve followed by Whatmann filter paper (No 1). The filtrate was then dried and the extract was stored in the refrigerator for subsequent analysis. The extract will henceforth be referred to as KPF (Koju Polyherbal Formulation).

2.2.2 Acute Toxicity Study of KPF

The oral median lethal dose (LD_{50}) of KPF was determined in rats according to the method of Lorke.[25] with slight modifications. The study was carried out in two phases. In the first phase, 9 rats were divided into 3 groups of 3 rats each and were treated with KPF at doses of 10, 100 and 1000mg/kg body weight respectively after which they were observed for 24 hours for signs of toxicity and/ or mortality. Based on the results of the first phase, 9 rats were again divided into 3 groups of 3 rats each and were also treated with KPF at doses of 1600, 2900 and 5000 mg/kg body weight respectively in the second phase. The rats were then monitored 24 h after treatment and for signs of toxicity and/or mortality. The median lethal dose (LD_{50}) of KPF was estimated based on the observations in the second phase.

2.2.3 Evaluation of KPF for Anti- hyperglycaemic Activity

Hyperglycaemia was induced in rats by the method described by Dunn and McLetchie.[26] Alloxan at a dose of 150mg/kg body weight was intraperitoneally administered to overnight fasted rats. The animals were thereafter allowed food and water *ad libitum*. Hyperglycaemia was confirmed in rats after 72 hours following an overnight fast using a Fine Test® digital glucometer and its corresponding test strip. Rats having fasting blood glucose (FBS) levels ≥ 200mg/ dl were considered hyperglycaemic and selected for the study. Thirty Wistar Albino Rats (30) (24 diabetic and 6 non- diabetic) were divided into 6 groups of 6 animals each and treated as follows: Group I: (non – hyperglycaemic control group) received normal saline (10ml/kg) Groups II to IV rats were hyperglycaemic and received 100, 200, 400 mg/kg KHF respectively while Group V rats were also hyperglycaemic and received 150 mg/kg Metformin. All treatments were done daily and orally for 28 days. Fasting Blood Sugar was monitored weekly using Fine Test® digital glucometer and its corresponding test strip.

2.2.4 Evaluation of KPF for Anti- Bacterial Activity

Antibacterial screening test

E. coli and *Salmonella spp* were subcultured on Brilliant Green Agar (BGA) and MacConkey agar, while *Staphylococcus* species was on sheep blood agar and incubated at 37°C for 24 h. Colonial morphology was observed and Gram's staining was carried out. 10 and 50% solutions of KHF were prepared by dissolving 1 and 5 g in 10 ml each of distilled water. 10 ml each of the prepared concentrations were pipetted into sterile test tubes. Bacterial aliquots of the test organisms were made by scooping 2 colonies each of a 24 h growth of the bacteria into 4 ml of sterile distilled water. 0.2 ml of each of the aliquots containing approximately 5 ×

104 bacterial cells or colony forming units was transferred into both of the extract concentrations and allowed to stand for an hour for reaction to take place between the extracts and the bacterial organisms. The mixtures were then inoculated on separate nutrient agar plates and incubated at 37°C for 24h.

Determination of the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and kill- time of Koju Polyherbal Formulation (KPF) on Bacterial isolates

The 50% Solution of KHF was chosen as the working concentration based on the observations from the antibacterial screening. It was subjected to double fold serial dilution in 3 sets of 7 test tubes each containing 5 ml of double strength nutrient broth. The nutrient broth served as diluent and media. 0.1 ml of each of the test organisms' aliquot was added to each of the serially diluted extracts and incubated at 37°C for 24 h. MIC was read as the concentration equivalent to the last test tube showing visibly complete clearance. Contents of 3 consecutively retrospective test tubes from the MIC test tubes were plated on nutrient agar and incubated at 37°C for 24 h. The concentrations of extract in the last plates that showed no growth were taken as the MBC. To obtain the Kill-time, a dilution of the extract using normal saline was made to the MBC equivalents obtained. 0.1 ml aliquot of each of the test organisms were added to the respective extract concentration and plated on nutrient agar at time intervals of 10 s, 20 s, 30 s, 1 min, 2 min, 5 min, 10 min, 20 min, 40 min and 60 min. This was to determine the contact time needed by the crude extract to kill the bacteria. The plates were incubated at 37°C for 24 h. The first plates that yielded no growth were recorded against their corresponding time.

2.2.5 Evaluation of Koju Polyherbal Formulation (KPF) for Anti- Malarial Activity

Thirty (30) albino mice were passaged intraperitoneally with standard inocula of 1×107 P. berghei infected erythrocytes. Seventy two hours after, the mice were randomly divided into 5 groups of 6 mice per cage. Group I served as normal control and received 0.2 ml of normal saline Groups II to IV received 100, 200 and 400 mg/kg KHF respectively while Group V received 10 mg/kg of Chloroquine diphosphate. All doses were administered orally and treatment continued daily until the seventh day when thin films were made from the tail blood of each mouse. The films were fixed with methanol, stained with Giemsa and parasitemia density examined by microscopically counting the parasitized red blood cells on at least 1 000 red blood cells in 10 different fields. The mean survival time of each group was

determined by finding the average survival time (days) of the mice in each group over a period of 28 days (D0-D27).

2.2.6 Evaluation of Koju Polyherbal Formulation (KPF) for Analgesic Activity

In determining the analgesic activity of the extract the writhing test was carried out according to the modified method of Yaksh.[27] The hot plate was made red hot; the animals were given the extract 30 min before each animal was placed into the hot plate and the first writhing was observed from the period of placing the animal on the hot plate to the time of writhing. This method was repeated for every 30 min for 3 consecutive times.

2.2.7 Evaluation of Koju Polyherbal Formulation (KPF) for Anti- inflammatory Activity

Acute inflammation was produced by injecting a fresh egg albumin into the plantar surface of rat hind paw according to a modified method of Winter.[28] KHF (100, 200 and 400 mg/kg, respectively) was administered 30 min before the fresh egg albumin injection. The paw volume was measured at 30, 60 and 90 min, respectively using the Archimedes principle and the difference in paw volume at 0 h were taken as a measure of oedema.

2.2.8 Evaluation of *Koju Polyherbal Formulation (KPF)* for Anti- pyretic Activity

The antipyretic activity of KHF in rats which were made hyperpyretic by injecting suspension of baker's yeast was investigated by a combination of methods described by Chatterjee[29] and kesersky.[30] pyrexia was induced in albino rats each, by subcutaneous injection of 50% dried baker's yeast suspension. Initial rectal temperature was recorded. After 18 h animal that showed a slight increase in rectal temperature were selected. KHF was administered to the rats (100, 200 and 400 mg/kg, respectively). The rectal temperature was measured by Ellab themostat at intervals of 30 min for 3 consecutive times after the extract administration.

2.3 Data and statistical analysis

All data were expressed as Mean ± SEM and statistical differences between means were determined by one- way ANOVA followed by Duncan's *post –hoc* test for multiple comparison tests using GraphPad version 5. 0. Values were considered significant at $P \leq 0.05$.

3.0 RESULTS

3.1 Acute toxicity test

During both phases of the study, the rats in all the groups did not show any sign of acute toxicity such as piloerection, lacrimation or changes in locomotion and respiration. Also, the highest dose of KPF (5000mg/ kg) administered to the rats produced zero mortality within the 14-day experimental period. The oral LD_{50} was therefore taken to be > 5000 mg/kg.

3.2 Anti- Hyperglycaemic Activity of Koju Polyherbal Formulation (KPF) in Wistar Rats

The FBS of the treated hyperglycaemic rats are presented in Table 1. KPF showed a dose- and time- dependent reduction in FBS. At a dose of 100mg/kg, KPF produced significant ($p<0.05$) reduction in FBS only on Day 28 while at a dose of 200mg/kg, KPF produced significant ($p<0.001$) reduction on days 21 and 28 when compared to the control. KPF (400mg/kg) produced statistically significant ($p<0.01$), ($p<0.001$) reductions in FBS on days 7 and 14, days 21 and 28 respectively. Metformin produced similar effect as 400mg/kg KPF.

Table 1: Effect of Koju Polyherbal Formulation (KPF) on Weekly Fasting Blood Sugar (FBS) of Hyperglycaemic Wistar Rats.

	Treatment Time in Days				
Treatment mg/kg	**0**	**7**	**14**	**21**	**28**
Control	386.0±07.83	374.5±11.21	358.5±11.32	380.0±13.12	370.5±13.16
KPF 100	369.8±12.11	352.8±13.12	320.3±11.24	335.3±09.03	291.5±14.03*
KPF 200	337.5±13.33	333.3±12.10	285.5±12.03	246.3±11.15***	223.1±11.09***
KPF 400	302.9±10.18	292.5±05.44**	241.3±10.33**	224.8±12.14***	214.5±12.15***
MET 150	368.8±14.18	287.9±13.11**	238.5±11.31**	226.3±16.17***	215.5±08.13***

Data represented as mean ± S.E.M (n=6), analysed by one- way ANOVA followed by Dunnett's *post – hoc* test for multiple comparisons. *p < 0.05, ** p < 0.01, ***p< 0.001, statistically significant reduction in FBS compared with the control

3.3: Anti- Bacterial Activity of Koju Polyherbal Formulation (KPF) (against *Escherichia coli, Staphylococcus aureus and Salmonella spp* isolates)

Table 2 shows that KHF at concentrations at the concentrations used had no inhibitory effect on *E .coli* and *Staphylococcus*. However, KHF produced zone of inhibition of 10 to 20 mm against *Salmonella spp*. As shown in Table 3, *E. coli* required the highest concentration (275 .0 mg/ml) of KHF to cause a bactericidal effect and also took the longest time of 20 minutes before the effect could be seen. *Staphylococcus* needed a concentration of 250 mg/ml for

bactericidal effect while *Salmonella* needed a concentration of 150 mg/ml. The kill time for *Staphylococcus* and *Salmonella* was 10 and 6 minutes respectively.

Table 2: Inhibitory Effect of Koju Polyherbal Formulation (KPF) on *Escherichia coli, Staphylococcus aureus* and *Salmonella spp* isolates by disc diffusion method.

Isolate	Concentration of Extract (mg/dl)	Zone of Inhibition (mm)	Interpretation
E. coli			
	1	0	Resistant
	3	0	Resistant
	5	0	Resistant
S. aureus			
	1	0	Resistant
	3	0	Resistant
	5	0	Resistant
Salmonella spp			
	1	10- 20	++
	3	10- 20	++
	5	10- 20	++

Table 3: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and kill- time of Koju Polyherbal Formulation (KPF) on Bacteria Isolates.

Isolate	MIC (mg/ ml)	MBC (mg/ ml)	Kill- time (sec)
E. coli	125.25	275.00	1200
S. aureus	175.50	250.75	600
Salmonella spp	75.50	150.00	360

3.4 Anti- Malarial Effect of Koju Polyherbal Formulation (KPF) in Mice

There was a dose dependent reduction in the level of parasitemia in the treated groups. On day 7 post- treatment, KPF at a dose of 100 mg/kg significantly ($p<0.05$) reduce the level of parasitemia. At 200 and 400 mg/kg KPF also significantly ($p<0.001$) reduce parasitemia density compared to the control. The mean survival time also increased dose dependently. Death was observed in the control group on day 8 and by day 12 all mice in the group died (mean survival time of 10.2 days). On the other hand, mice in the group that received 100, 200 and 400 mg/kg survived beyond 21 days. Chloroquine treated group survived the 28 days duration of observation (Table 4).

Table 4: Effect of Koju Polyherbal Formulation (KPF) on P. berghei berghei in mice.

Drug	Dose mg/kg	MPD Pre (D3)	Post (D7)	Mean Survival Time (Days)
Normal Saline	Control	30.0±0.8	37.5±0.5	10.2±1.2
KPF	100	29.3±0.1	25.8±0.2*	23.3±1.4
KPF	200	27.5±0.3	13.3±0.1***	25.5±1.3
KPF	400	29.9±0.8	5.2±0.4***	27.3±1.3
Chloroquine	10	29.8±0.3	3.9±0.1***	28.0±0.0

MPD= Mean Parasitemia Density, D3=Day three, D7=Day seven, Data represented as mean ± S.E.M (n=6), analysed by one- way ANOVA followed by Dunnett's *post – hoc* test for multiple comparisons. *p < 0.05, p < 0.01, ***p< 0.001, statistically significant reduction in parasitemia compared with the control. All mice treated with Chloroquine survived until day 28.**

3.5 Analgesic Effect of Koju Polyherbal Formulation (KPF) in Wistar Rats

As shown on Table 5, KPF at all the doses administered, produced time dependent suppression of nociceptive response in the rats. All the doses of KPF and the standard drug- Aspirin after 30 minutes of administration produced significant ($p<0.05$) suppression of nociceptive response in the rats compared to the control. Similarly, at 60 minutes, KPF at all the doses administered produced significant ($p<0.01$) suppression of nociceptive response in the rats except the Aspirin- treated group ($p<0.05$). At 90 minutes post- treatment, KPF and Aspirin produced similar significant ($p<0.001$) suppression of nociceptive response in the rats compared to the control.

Table 5: Effect of Koju Polyherbal Formulation (KPF) on Analgesic Response Induced by Hot Plate.

Treatment mg/kg	Reaction Time (Sec) 30 min	60 min	90 min
Control	3.25±0.12	4.20±0.17	3.70±0.86
KPF 100	3.30±0.24	10±0.93**	12.5±0.43***
KPF 200	6.51±0.23*	11.3±0.75**	13.7±0.29***
KPF 400	5.81±0.33*	9.8±0.44**	11.5±0.15***
Aspirin	5.35±11.1*	7.3±0.39*	9.5±0.73***

Data represented as mean ± S.E.M, *p < 0.05, ** p < 0.01, ***p< 0.001, statistically significant compared with the control

3.6 Anti- Inflammatory Effect of Koju Polyherbal Formulation (KPF) in Wistar Rats

KPF only at a dose of 400 mg/kg produced significant ($p<0.05$) inhibition in the paw edema when compared to the control. The standard anti-inflammatory drug Aspirin produced similar significant ($p<0.05$) inhibition at the later phase (2 h) of paw volume increase.

Table 6: Effect of Koju Polyherbal Formulation (KPF) on Egg Albumin-induced Rat Paw Oedema.

Treatment mg/kg	Reaction Time (Sec) 30 min	60 min	90 min	120 min
Control	2.59±0.25	1.88±0.23	1.80±0.04	1.37±0.03
KPF 100	2.52±0.72	1.93±0.28	1.73±0.33	1.43±0.53
KPF 200	2.38±0.18	1.86±0.43	1.86±0.15	1.33±0.49
KPF 400	2.92±0.19	1.83±0.39	1.79±0.54	0.83±0.05*****
Aspirin	2.34±0.11	1.22±0.41	1.21±0.69	0.89±0.13*****

Data represented as mean ± S.E.M, $p < 0.05$, statistically significant compared with the control

3.7 Anti- Pyretic Effect of Koju Polyherbal Formulation (KPF) in Wistar Rats

As shown in Table 7, KPF at all the doses administered (100, 200 and 400 mg/kg) did not produce a significant decrease in the temperature elevation induced with yeast.

Table 7: Effect of Koju Polyherbal Formulation (KPF) on Baker's Yeast-induced Pyrexia in Rats.

Treatment mg/kg	Rectal temp. (^{0}C) Temp before induction	18h after induction	Rectal temp. after drug administration (^{0}C) $18^1/_2$ h	19 h	$19^1/_2$ h
Control	37.00±0.85	37.50±0.13	37.65±0.12	37.340±0.21	37.50±0.26
KPF 100	36.90±0.17	38.81±0.12*	37.63±0.14	37.45±0.03	37.40.5±0.03
KPF 200	37.05±0.63	39.30±0.31*	37.58±0.23	37.63±0.16	37.57.1±0.09
KPF 400	37.90±0.28	39.50±0.48*	37.60±0.13	37.58±0.19	37.49.5±0.09
Aspirin	38.09±0.41	38.99±0.63*	37.75±0.16	37.39±0.21	37.50±0.113

Data represented as mean ± S.E.M, *$p < 0.05$ statistically significant compared with the control

4.0 DISCUSSION

Herbal medicines are now receiving greater attention as an alternative to clinical therapy leading to increase in their demands.[31] In the rural communities of developing countries, the exclusive use of herbal drugs to treat various diseases is still very common and is prepared most often and dispensed by herbalists without formal training. Experimental screening method is therefore important in order to establish the active components present, ascertain the efficacy and safety of the herbal products.[32]

In the acute toxicity study of KPF, no noticeable changes in the behavior and in the nervous system responses were observed in the treated animals. All the rats that received 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight survived beyond the 2- week period of observation, therefore, suggesting the median acute toxicity value (LD_{50}) of KPF to be above 5000 mg/ kg body weight. According to Ghosh[33] and Klaasen[34], KPF can be classified as being non- toxic, since the LD_{50} by oral route was found to be above 5000 mg/kg body weight.

As shown in Table 1, KPF exhibited time and dose- dependent anti- hyperglycaemic effect having lowered significantly the plasma sugar levels supporting the claim of the marketers of the polyherbal formulation that it can be useful in the management of diabetes. This anti-hyperglycaemic activity are likely due to the high flavonoid content of the individual plants as flavonoids have been linked with anti- hyperglycaemic activity. Quercetin and kaemferol have been shown to be antioxidant and hence anti- hyperglycaemic in nature.[35] Hypoglycaemic activity of kaemferol derivatives from many medicinal plants has been reported by Desoky and Yousef.[36] These antioxidants might have played a role in scavenging the free radicals generated by alloxan leading to the regeneration of the beta-cells destroyed by alloxan, hence an increase in release of insulin and reduction in glycaemia.[37] KPF might have also produced anti-hyperglycaemic activity through direct release of insulin by inhibiting the ATP- sensitive potassium channels in the membrane of the residual beta-cells just like sulfonylureas and meglitinides. It is also possible that KPF might have potentiated the action of insulin to stimulate glucose uptake and utilization by tissues, especially by the liver, skeletal muscle, and adipose tissue.[38]

The result of the antibacterial screening tests of KPF carried out on the selected bacterial isolates (*E. coli*, *S. aureus and Salmonella* spp) is shown in Table 2. KHF at the concentrations used had no inhibitory effect on *E .coli* and *Staphylococcus.* However, KHF

produced zone of inhibition of 10 to 20 mm against *Salmonella spp.* It is important to state here that the Clinical and Laboratory Standard Institute (CLSI 2010) method for interpreting MIC was not applied here owing to the fact that we were dealing with crude extracts here while the CLSI system deals in purified active ingredients. So because there are quite a number of other ingredients in these crude extracts we could not determine the concentration of the active ingredients. Therefore our measurements here were in mg instead of µg. As shown in Table 3, *E. coli* required the highest concentration (275.0 mg/ml) of KHF to cause a bactericidal effect and also took the longest time of 20 minutes before the effect could be seen. *Staphylococcus* needed a concentration of 250 mg/ml for bactericidal effect while *Salmonella* needed a concentration of 150 mg/ml. The kill time for *Staphylococcus* and *Salmonella* was 10 and 6 minutes respectively. The difference in the effect of this plant extracts within the organisms suggested that there are different antibacterial compounds in the plant extracts and that the compound that acted on one may not be the same as the one that acted on the others since antibacterial agents have different modes of action.[39] This phenomenon of varied susceptibility was also observed by Ergene.[40]

In this study, Chloroquine was used as the standard antimalarial drug. Chloroquine has been used for curative, suppressive and prophylactic antiplasmodial activities. In early and established infection, Chloroquine interrupts with the heme polymerization by forming a FP-Chloroquine complex. This complex is responsible for the disruption of the parasite's cell membrane function and ultimately leads to auto digestion. Though, Chloroquine exhibited higher curative antiplasmodial activities by the extent of inhibition of parasitemia, KPF at 200 and 400 mg/kg showed similar antiplasmodial activities. P. berghei berghei has been used in studying the activity of potential anti-plasmodials *in vivo* in rodents[41], [42] and it produces diseases similar to those of human plasmodial infection.[43], [44] This study, as will be discussed in the next paragraphs showed that KPF possess analgesic properties. Agents with such properties are known to produce additional remedy to malaria patients.[45]

Table 5 showed that KPF at all doses after 90 minutes produced significant ($p<0.001$) suppression of nociceptive response. HPF is comparable to Aspirin in this effect. KPF might have exerted its analgesic activity by inhibition of some inflammatory response as analgesic could be due to inhibition of sensory receptor stimulation or due to anti inflammatory action.[46]

In Table 6, KPF at 400 mg/kg produced an inhibition in the paw edema. Like the standard anti-inflammatory drug Aspirin the inhibition was at the later phase of paw volume increase. The paw edema model is a standard method used for evaluation of anti-inflammatory activity of anti-inflammatory agents including several mediators of inflammation such as prostaglandins, serotonin, histamine and bradykinin.[47], [48]

The evaluation of the antipyretic effect of KPF revealed that the polyherbal formulation did not have significant effect on the temperature induced experimentally with yeast. The yeast induced fever is a well-established model for assessing antipyretic effect and it has been used in a number of studies.[49] Generally antipyretic activity is exerted by inhibition of prostaglandin synthesis via cyclo- oxygenase activity.[50] With the failure of KPF to produce significant changes in the elevated temperature, it could be that KPF has no inhibitory effect on cyclo- oxygenase.

5.0 CONCLUSION

It can be concluded that anti-hyperglycaemic, anti- bacterial, anti- malarial, analgesic and anti- inflammatory activities are part of the pharmacological profile of Koju polyherbal formulation.

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