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ASSESSMENT OF GENETIC AND REPRODUCTIVE TOXICITY OF TONGKAT ALI PLUS®

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ABSTRACT

Tongkat Ali Plus® is a mixture of *Eurycoma longifolia* Jack and *Lepidium meyenii* powder as active ingredients. The objective of the present study is to evaluate the potential genotoxicity (in vivo &in vitro) and developmental toxicity of Tongkat Ali plus®. Bacterial reverse mutations assay was performed in *Salmonella typhimurium* strains and Escherichia coli (WP2-uvrA). In addition, chromosome aberrations and micronucleus assays were studied to investigate the *in vivo* genotoxicity in bone marrow cells of rats. Tongkat Ali plus® treated female and male rats were mated to evaluate the reproductive toxicity of Tongkat Ali plus® on female and developmental toxicity on their offspring. Results revealed that Tongkat Ali Plus® did not cause neither *in vitro* genotoxicity (at dose levels tested were 1750, 875, 175, 87.5,43.7 and 17.5 µg/plate) nor *in vivo* genotoxicity (at dose levels 250, 500 and 1000 mg/kg for consecutive 14 days). In addition, Tongkat Ali Plus® did not show obvious alteration in the reproductive performance or fetal development (at dose levels 1000, 375, 125 mg/kg for 14 days). Based on these endings, we can conclude that the use of Tongkat Ali Plus® in traditional medicine poses no risk.

KEYWORDS: **Tongkat Ali Plus®, genotoxicity, Bacterial reverse mutation, chromosome aberrations, Micronucleated polychromatic erythrocytes, reproductive toxicity.**

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INTRODUCTION

Herbal-based phytochemical industry is a new and upcoming industrial sector in Malaysia. One of the most important phytochemicals in the Malaysian market is *Eurycoma longifolia* that commonly known as Tongkat Ali. It is traditionally use for its aphrodisiac, anti-pyretic and anti-malarial effects.[1]*Eurycoma longifolia* has a range of medicinal properties as a general health tonic, including improvement in physical and mental energy levels and overall quality of life. [2] The roots of Tongkat Ali, called Malaysian ginseng and are used as a traditional anti-aging remedy to help older individuals adapt to the reduced energy, mood, and libido that often comes with age. [3]*Eurycoma longifolia* contains a group of small peptides referred to as "eurypeptides" that are known to have effects in improving energy status and sex drive in studies of rodents. [4,5] The effects of Tongkat Ali in restoring normal testosterone levels appears to be less due to actually stimulating testosterone synthesis, but rather by increasing the level of free testosterone from its binding hormone, sex-hormone-binding-globulin.[6] Therefore, *Eurycoma longifolia* supplementation may be an alternative for testosterone replacement therapy and the bone volume restoration from androgen deficient osteoporosis [7] or it may be able to improve certain mood state parameters in elders. [8] *Lepidium meyenii* (maca) is a Peruvian plant of the Brassicaceae family with high potential for bio-prospecting. Maca has been used for centuries in the Andes for nutrition and to enhance fertility.[9] Maca is used as a food supplement for its medicinal properties described traditionally. There are no reports of adverse reactions after consuming *Lepidium meyenii* in food.[10] The hypothesis that maca may be effective in improving health status, particularly reproductive function, is supported by several lines of evidence. Historical aspects and biological properties of maca, gathered from experimental and clinical studies on this species reveal the importance of this plant as nutraceuticals food. [9, 11] Maca seems to be promising as a positive influence on chronic human diseases characterized by high lipid levels in the blood, inadequate control of free radicals that can lead to DNA damage and high blood sugar levels.[12]*Eurycoma longifolia* has been mixed with other reputedly nontoxic herbs in many herbal products preparation for improving genera health rather than to improve strength and power during sexual activities alone.[13] However, mixing of *Eurycoma longifolia* with other herbs may increase the chances of *Eurycoma longifolia* based herbal products being contaminated with toxic materials such as heavy metals and it may change the cytotoxicity status due to combination of various plants metabolites. In addition, although herbal medicines rely on remedies of natural origin and have fewer side effects than allopathic medicines, the use of herbal remedies is not always safe. Hence, concerns about their safety and toxicity are increasing especially within the context of chronic or cumulative dosing.[14] Therefore, the objective of the present study was to evaluate the *in vitro* and *in vivo* genotoxicity and reproductive toxicity of Tongkat Ali plus®. The *in vitro* genotoxicity was conducting using Ames bacterial reverse mutation assay. The bone marrow chromosome aberration and the erythrocyte micronucleus assays were carried out in Sprague-Dawley male and female rats to evaluate *in vivo* genotoxicity. In addition, reproductive toxicity study was conducted to evaluate the effect of Tongkat Ali Plus®administration on the pregnant females' reproductive performance and their fetuses. All studies were performed following standard protocols, as recommended by the testing guidelines of the Organization for Economic Cooperation and Development (OECD) 471, 474, 475 and 421.

MATERIALS AND METHODS

Drug

Tongkat Ali Plus® hard gelatin vegetable origin capsule consist of 200 mg Tongkat Ali powdered extract from *Eurycoma longifolia* Jack powdered and 100 mg Maca powdered extract from dried tuberous root of *Lepidium meyenii* as active ingredients. 100mg of starch 1500 (inactive ingredient) was used as diluents. The extracts were obtained from TPM Biotech Sdn Bhd, Kuala Lumpur, Malaysia.

Ames Bacterial Reverse Mutation Assay

Salmonella typhimurium strains (TA 98, TA100, TA 97a, and TA 1535) and Escherichia coli (WP2-uvr A) were used for test. Test was conducted by metaphase analysis both in the absence and in presence of S9 fraction according to OECD 471 (The OECD guideline for testing of chemicals in a Bacterial Reverse Mutation Test) under GLP complaint facility (TetraQ, Australia; GLP No. 15153). In the case of absence S9, Sodium azide (NaN_3), 9-aminoacridine (9-AA), 4-nitroquinoline 1-oxide (4-NQO), and 2-aminoanthracene (2-AA) were used as positive controls and in the case with S9 fraction, 2-AA was used. Each 5 strain was put in a test tube and blended with 0.1 ml fermentation solution, 0.05 ml AMA-0 and 0.5 ml phosphate buffer saline(0.2M, pH 7.4) (0.5 ml S9 mix in case of metaphase analysis) and then pre-incubated at 37°C for 30 min. After the pre-incubation, 2 ml top agar was added and double lay on minimal glucose agar plate, and then incubated at 37°C for 48 hr. Reverse mutated colonies were counted. When the reverse-mutated colonies increase dose dependently or more than twice, it is positive as mutagen.[15] The dose levels tested were 1750, 875, 175, 87.5, 43.7 and 17.5 µg/plate.

Animals

Fifty Sprague-Dawley male and female rats (25♀, 25♂) with a body weight ranging from 120 to 150 g for both sexes were obtained from Misr University for Science and Technology, 6th of October, Egypt. The animals were acclimated for a period of one week before the beginning of the experiments. Rats were maintained under controlled of temperature (22±3°C), 50-55% relative humidity and light cycle of 12 h light: 12 h dark and were fed standard granulated diet and water *ad labium.* This study was conducted at faculty of pharmacy animal facility; Ain shams University in compliance with the OECD good laboratory practice principles and applicable standard operating procedures. Rats of both sexes were randomly divided

into five groups of five rats per group. Negative control group: animals administered orally distilled water at dose (10 mL/ kg b. w.). Positive control group: animals were injected intraperitoneally (IP) with a single dose of 50mg/kg Cyclophosphamide. Tongkat Ali plus® groups: three groups of rats were administered orally Tongkate Ali plus at three dose levels (250, 500 and 1000 mg/kg) for consecutive 14 days. The Animal Care and Use Committee of faculty of pharmacy approved all experimental protocols related to the use and care of laboratory animal in research.

Mammalian Micronucleus Test

The micronucleus assay was performed in accordance with the organization for economic cooperation and development (OECD) guideline 474. The bone marrow were extracted, washed with 500 µl fetal calf serum (FCS) and then centrifuged (160 ×g) for 5 min. After discard of upper layer, the erythrocytes were resuspended with FCS. The solution was smeared, dried and fixed with absolute methanol for 5min. The fixed sample was dyed with 5% phosphate buffered Giemsa (pH 6.8) for 5min, covered with a cover glass, then bone marrow cell proliferation, and micronucleated polychromatic erythrocytes (MNPCEs) were observed. To determine the proliferating rate, about 200 of total erythrocyte (polychromatic and normochromatic erythrocytes) selected in good smeared area were observed and the ratio of PCE/NCE were calculated. MN cells were counted from about 2,000 Of PCE to check out the MN frequency. The micronucleus assay was performed in accordance with the organization for economic cooperation and development guideline (OECD 474).

Chromosomal Aberration Analysis

At the end of treatment, schedule the animals of each experimental group were injected IP with 5 mL/kg b.w. of 0.5% colchicine as a metaphase arresting substance. Two hours after injection animals were sacrificed by dislocation of nick vertebra for preparation of bone marrow cells chromosomes according to the method of Yosida and Amano.[16] Bone marrow cells were collected from both femurs by flushing in saline solution and then incubated in hypotonic solution (kcl 0.56%) at 37°C for 30min, fixed in carnoy"s fixative. The cells were resuspended in small volume of fixative; dropped onto clean chilled slid, flam dried and stained with 10% buffered Giemsa (pH 6.8). One hundred good metaphase spreads from bone marrow of each animal were examined microscopically to detect the different types of chromosomal abnormalities.[16]

Developmental toxicity Assay

Male and female Sprague-Dawley 6–8 week-old rats were obtained from Misr University for Science and Technology, 6th of October, Egypt and allowed to acclimatize for at least two week prior to assignment to the studies. Dry food rodent pellets and water were provided *ad libitium* with animal house conditions maintained at 20-22°C and 65–70% relative humidity. Four groups each of 20 males and females; one serves as control and the other groups were treated with Tongkat Ali Plus®at three dose levels 1000, 375, 125 mg/kg for 14 day prior to and during mating. Pregnant females were continuing treatment through gestation, followed by treatment of pups to the 4th day of lactation in accordance with guidelines (OECD, 42). Individual body weights and Food consumption were measured on day 0, 7, 14 and 20 during the gestational phase of the developmental toxicology study. Gravid uterine weight was recorded and, beginning at the distal end of the left uterine horn, the location of viable and nonviable fetuses and early and late resorptions along each uterine horn and the total number of implantations were recorded. The numbers of ovarian corpora lutea were counted and following opening of the uterine horns, placentas were examined grossly in situ and fetuses collected.

Statistical Analysis

All data obtained in this study were expressed in mean ± standard deviation (SD). Comparisons between groups were performed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests by IBM SPSS software statistical (version 20). Difference between groups was considered statistically significant if ($P \leq 0.05$).

RESULTS

1- In Vitro Ames Bacterial Reverse Mutation test

The results of mutagenicity test are presented in Table1. No positive mutagenic responses of Tongkat Ali Plus® were observed with any of the tested strains of Base Pair Substitution bacterial strains TA 100 and WP2uvrA and Frame Shift mutation bacterial strains in either the presence or absence of S9 activation under the conditions of this study. Nevertheless, signs of positive mutagenic responses were observed with the tested strain TA 1535 with concentration of 875 and 1750µg/ plate.

2-In Vivo Micronucleus

Table 2 summarize the results of the analysis of micronucleus in bone marrow cells of Sprague-Dawley rats both sexes following treatment with different concentrations of the Tongkat Ali plus® extract and controls. Results showed that the administration of Tongkat Ali Plus® extract showed non-significant increase in the frequencies of micronucleated polychromatic erythrocytes as compared to negative control. On the other hand, cyclophosphamide resulted in significant ($p \leq 0.05$) increase in the number of micronuclei in polychromatic erythrocytes (MNPCEs) compared with the control group.

3-In Vivo Chromosomal Aberrations

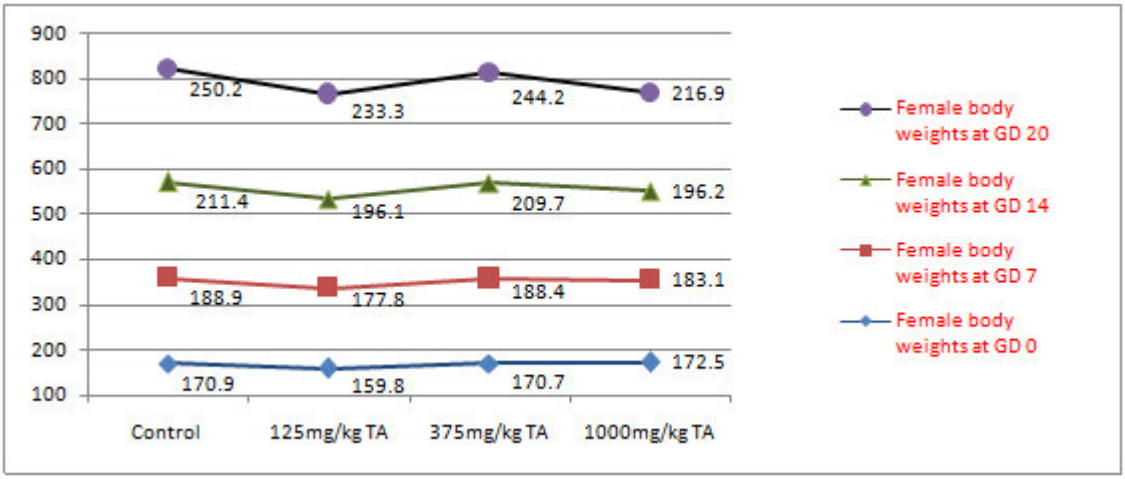
The results of *in vivo* chromosomal aberration study in both sexes of Sprague-Dawley rat treated with Tongkat Ali Plus® at three different doses are illustrated in Tables 3 and 4. Tongkat Ali Plus® showed no significant increase in the frequencies of individuals and total chromosomal aberrations in bone marrow cells of rat of either sex when compared with vehicle control group. Whereas, cyclophoshamide has significantly increased ($P \leq 0.001$) the frequencies of different types of chromosomal aberrations in animals of both sexes.

4-Developmental Toxicology

The data showed there was no significant effect on the body weights of pregnant females treated with Tongkat Ali plus® dosage of 375 mg/kg. While, Tongkat Ali Plus® at doses of 125 and 1000mg/kg were significantly decreased the pregnant female body weights at gestation day 14 and 20 as compared to control group (Figure 1).

Graph 1

Pregnant female body weight change in control and Tongkat Ali Plus® treated pregnant females

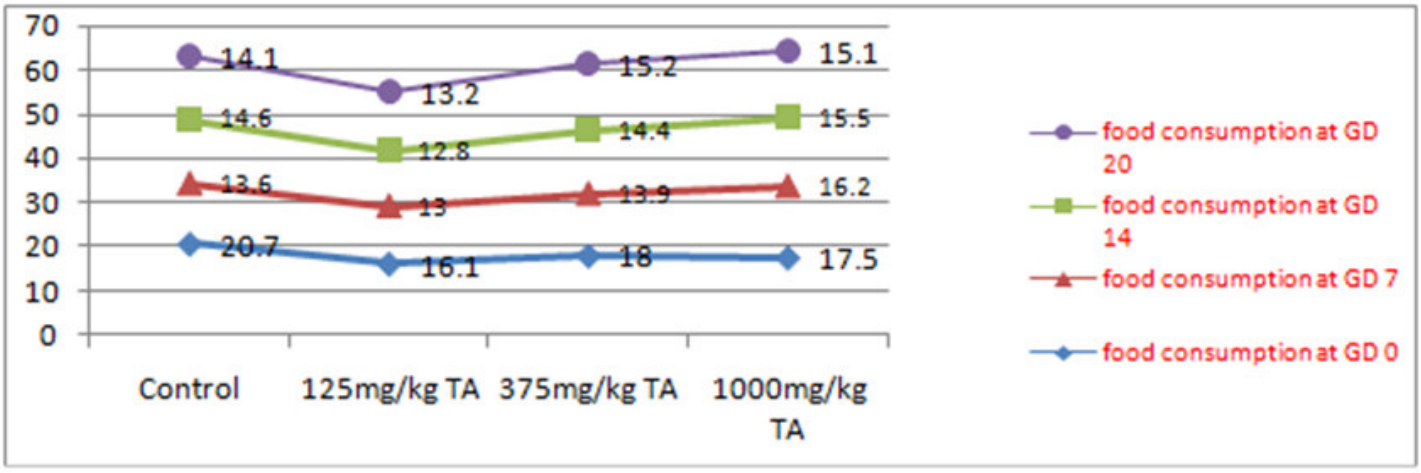


Food Consumption

There was no significant change in pregnant females' food consumption in Tongkat Ali Plus® and control groups through the gestation period as show in (Figure 2).

Graph 2

Food consumption in Tongkat Ali Plus® and control pregnant female groups



Reproductive performance

The results of female reproductive performance revealed that there were no effects of Tongkat Ali Plus® treatment on the potency of Sprague-Dawley rats.Whereas, the high dose decreased the percent of positive pregnancy to (55%) as compared to control (80%) as shown in Table 5. Tongkat Ali Plus® induced dose dependent increase in the female pregnancy duration to ≥ 23 days. In addition, Tongkat Ali Plus® high dose decrease the percent of dams with live born at 0 and 4 days to (70 and 57.1%) as compared to control (93.8 control (93.8 and 100%)respectively. The administration of Tongkat Ali Plus® pre-mating and during pregnancy at the three dosages has not significantly changed the percentage of number of pubs, pre- and post-implantation losses, and litter size at zero days (Table 6). Whereas, the high dose significantly decreased ($p= 0.01$) the pubs numbers and weights at 4day of lactation when compared to control and other treated groups.

Table 1

Mutagenicity assay for Tongkat Ali Plus® with and without-metabolic activation using S. typhimurium and E. coli strains

Compound	Concentration of test material (µg/plate)		Average of revertants colonies (Mean +SD)				
			Base Pair Substitution			Frame Shift mutation	
			TA 100	TA 1535	WP2 uvrA	TA 98	TA 97a
Historical negative (background)			16.00+1.00b	6.66+0.57c	12.00+1.00b	31.66+ 1.53b	26.00+1.00b
Tongkat Ali Plus	1.0	-S9	0.19+0.01e	0.33+0.03f	0.08+0.03c	0.06+0.02d	0.07+0.02e
		+S9	0.31+0.03e	0.45+0.05c	0.21+0.07c	0.24+0.05dd	0.22+0.01d
	2.5	-S9	0.85+0.04e	2.16+0.02e	0.54+0.01c	0.16+0.02d	0.16+0.02e
		+S9	0.97+0.03d	2.28+0.02c	0.64+0.01c	0.26+0.01d	0.26+0.02d
	5.0	-S9	1.48+0.06d	3.03+0.05d	0.66+0.04c	0.48+0.02d	0.32+0.02e
		+S9	1.65+0.08d	3.25+0.13c	0.84+0.09c	0.71+0.10d	0.47+0.11d
	10	-S9	6.02+0.06c	3.91+0.08d	0.87+0.05c	0.94+0.04d	0.63+0.03e
		+S9	7.02+0.11c	4.08+0.07c	1.10+0.13c	1.12+0.02d	0.86+0.08d
	50	-S9	6.04+0.08c	16.01+0.01b	1.10+0.04c	3.15+0.05c	1.83+0.03d
		+S9	7.37+0.41c	17.68+0.4b	1.27+0.08c	3.98+1.75c	2.40+0.36c
	100	-S9	6.02+0.06c	16.00+0.0b	8.68+0.03b	3.17+0.07c	2.98+0.02c
		+S9	7.38+0.48 c	17.55+0.27b	9.76+0.13b	4.25+0.25c	3.70+0.26c
Positive control			NaN3	NaN3	ENU	2NF	9AA
			88.00+1.00a	29.66+1.9a	93.02+6.12 a	71.67+1.53a	83.00+1.7a

Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

Table 2

Frequencies of micronucleated polychromatic erythrocytes in bone marrow cells of rats treated with Tongkat Ali Plus® for 14 days

Treatment	Dose mg/kg body weight	No of micronucleated polychromatic erythrocytes M±SD		Micronucleated polychromatic erythrocytes % M±SD	
		♂	♀	♂	♀
Negative Control	0	8.20±1.48b	8.80±0.83b	0.41±0.07b	0.44±0.04b
Positive Control	50	35.20 ±9.20a	38.20 ±9.12a	1.91±0.45a	1.76±0.46a
Tongkat Ali Plus	250	8.60 ±1.14b	9.00 ±1.00b	0.43±0.05b	0.45±0.05b
	500	9.00 ±1.22b	9.20 ±0.83b	0.45±0.06b	0.46±0.04b
	1000	9.80 ±0.83b	10.00 ±0.70b	0.49±0.04b	0.50±0.30b

Two thousand cells were analyzed per animal, for a total of 20000 cells per group. Data were expressed as Mean ± SD.
Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

Table 3

Chromosome aberrations induced in bone marrow cells of male rats treated with Tongkat Ali Plus® for 14 days

Items		-ve control	+ve control	TA250mg/kg	TA500mg/kg	TA1000mg/kg
Numerical chromosomal aberrations	n-	2.40±1.14 a	3.60±0.54 a	1.80±0.44 a	2.00±0.70 a	1.60±0.55 a
	n+	0 b	1.60±0.55 a	0.40±0.55 b	0.60±0.55 b	0.67±0.54 ab
	Polyploidy	1.00±0.02 b	2.40±0.89 a	0.75±0.50 b	1.00±0.05 b	1.20±0.44 b
	Total	3.40 ±1.14 b	7.60±1.14 a	2.80±1.09 b	3.60±0.89 b	3.20±0.83 b
Structural chromosomal aberrations	Dicentric	0.60 ±0.54 b	2.00±1.22 a	0.40±0.54 b	0.40±0.55 b	0.80±0.44 b
	CF	0 c	2.00±0.70 a	1.00±0.50 b	1.25±0.50 b	1.25±0.50 b
	Ring	0 d	1.80±0.44 a	0.67±0.57 cd	1.00±0.00 bc	1.50±0.70 ab
	Break	2.00±0.70 b	4.40±1.67 a	1.60±0.55 b	1.50±0.58 b	1.60±0.54 b
	Chr gap	0 a	0.60±0.55 a	0.40±0.54 a	0.40±0.54 a	0.40±0.54 a
	Cht gap	2.00±0.71 b	3.20±0.83 a	1.40±0.55 b	1.40±0.54 b	1.60±0.55 b
	Del	0 c	3.00±1.41 a	1.00±0.48 bc	1.25 ±0.50 b	1.33±0.58 b
	End to end	0 c	2.20±0.84 a	0.80±0.83 bc	1.00±0.25 b	1.25±0.50 b
	CA	3.00 ±0.71 b	6.00±0.71 a	2.50±0.58 b	2.20±0.84 b	2.20±0.84 b
	Fragment	1.60 ±0.53 b	3.40±1.81 a	1.20±0.83 b	1.20±0.44 b	1.25±0.50 b
	Total	9.20 ±1.09 b	28.60±1.81 a	10.20±1.30 b	10.00±1.00 b	11.00±1.00 b
Total chromosomal aberrations excluding gap		10.00±1.22 b	31.00±5.33 a	9.00 ±1.58 b	8.40±1.34 b	9.40±1.14 b
Total chromosomal aberrations including gap		12.60±0.54 b	36.20±2.16 a	13.00±1.00 b	13.60±0.89 b	13.80±0.44 b

Data are presented as Mean ± S.D. Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

Table 4
Chromosome aberrations induced in bone marrow cells of female rats treated with Tongkat Ali Plus® for 14 days

Items	-ve control	+ve control	TA250mg/kg	TA500mg/kg		TA1000mg/kg
Numerical chromosomal aberrations	**n-**	2.00±0.70 a	2.20±0.84 a	2.20±0.45 a	2.00±0.71 a	1.80±0.84 a
	n+	0 b	1.50±0.58 a	0.60±0.54 b	0.20±0.45 b	0.80±0.84 ab
	Polyploidy	1.00±0.48 b	2.20±0.84 a	0.67±0.58 b	1.00±0.10 b	1.25 ±0.50 b
	Total	2.60±0.89 b	5.60±0.55 a	3.20±0.85 b	3.00±0.71 b	3.40±0.55 b
Structural chromosomal aberrations	**Dicentric**	0.80 ±0.44b	2.00±0.71 a	0.50±0.57 b	0.67±0.58 b	0.60 ±0,89 b
	Centric Fusion	0 c	2.75±0.50 a	1.00±0.01 b	1.00±0.03 b	1.00±0.05 b
	Ring	0 b	1.40±0.55 a	1.00±0.11 a	0.67±0.58 ab	1.00±0.03 a
	Break	1.80±0.44 b	3.60±0.95 a	1.00±0.31 b	1.40±0.55 b	1.40±0.54 b
	Chr. gap	0 a	0.60±0.54 a	0.40±0.55 a	0.20±0.45 a	0.40±0.55 a
	Cht. gap	2.00±0.05 b	3.00±0.71 a	1.60±0.54 b	1.60±0.55 b	1.60±0.58 b
	Deletion	0 c	2.50±0.58 a	1.33±0.57 b	1.00±0.00 b	1.33±0.58 b
	End to end	0 c	2.60±0.89 a	1.00±0.02 b	1.33±0.57 b	1.00±0.37 b
	CA	2.60±0.54 b	5.20±0.84 a	2.60±0.55 b	2.50±0.58 b	2.50±0.58 b
	Fragment	1.00±0.54 b	2.20±0.83 a	1.20±0.44 b	1.00±0.70 b	1.25±0.50 b
	Total	8.20±0.83 b	24.80 ±3.11 a	9.20 ±0.83 b	9.00 ±0.70 b	9.40 ±0.89 b
Total chromosomal aberrations excluding gap		8.80±0.84 c	27.00±2.82 a	10.60±0.89bc	10.40±0.54 bc	11.00±0.870 b
Total chromosomal aberrations including gap		10.80±0.83 b	30.40±3.11 a	12.20±0.84 b	11.80±0.45 b	12.80±0.84 b

Data are presented as Mean ± S.D. Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

Table 5
Effects on female's reproduction performance in control and Tongkat Ali Plus® groups

Parameter		Control		125mg/kg TA		375mg/kg TA		1000mg/kg TA	
		F	%	F	%	F	%	F	%
Positive pregnancy		16/20	80	16/20	80	18/20	90	11/20	55
Pregnancy Duration	≤ 21 days	1/15	6.7	2/14	14.3	0/17	0	0/8	0
	=22 days	14/15	93.3	11/14	78.6	13/17	76.5	3/8	37.5
	≥ 23 days	0/15	0	1/14	7.1	4/17	23.5	5/8	62.5
Dams with live born at day 0		15/16	93.8	17/18	94.4	14/16	87.5	7/10	70
Dams with dead born at day 0		1/16	6.3	2/16	12.5	1/18	5.6	3/10	30
No of live born at day 4		15/15	100	14/14	100	17/17	100	4/7	57.1
No of dead born at day 4		0/15	0	0/14	0	0/17	0	3/7	42.9

Qualitative parameters are expressed as frequencies and percentages.

Table 6
Developmental toxicity in control and Tongkat Ali Plus® pregnant female rats

Treatments	Pups number	Pre-implantation loss	Post-implantation loss	Live number at day		Litter weights at day	
				0	4	0	4
Control	7.80+0.42a	2.13+0.27a	0.73+0.47a	7.73+0.43a	7.73+0.43a	45.60+2.68a	71.19+4.32a
TA 125mg/kg	6.93+0.35a	1.33+0.28a	1.47+0.52a	6.73+0.35a	6.33+0.57a	41.37+2.52a	62.76+3.23a
TA375mg/kg	7.60+0.43a	1.20+0.31a	1.00+0.48a	6.93+0.68a	6.93+0.68a	44.73+2.74a	70.21+4.29a
TA1000mg/kg	8.13+0.33a	2.13+0.42a	1.73+0.74a	6.80+0.65a	4.00+0.89b	40.00+1.85a	39.87+9.34b

Data were expressed as Mean±SE. Mean values followed with different letters within the same column are significantly different from one another (P≤0.05).

DISCUSSION

Natural products have been the source of most active ingredients in traditional medicines that used for the treatment of diseases as well as the sources of numerous prescription and over-the-counter WP2uvrA and Frame Shift mutation bacterial strains in either the presence or absence of S9 activation. Nevertheless, signs of positive mutagenic responses were observed with the strain TA 1535 with concentration of 875 and 1750µg/ plate. Several studies confirmed that *Eurycoma*[21, 22]therapeutics. However, the quality control for the growth *longifolia* not mutagenic,since, *Eurycoma* and isolation of most traditional medicines is poor or *longifolia* aqueous extract had shown no toxicity[23] nonexistent. Patients and practitioners alike need to be confident of the quality, safety of traditional medicines.[17] Indeed; different studies have shown that several plants towards tested bacterial strains. Ming et al. indicated that *Eurycoma longifolia* aqueous extract was not mutagenic in the Salmonella/microsome assay. In®used in medical practice may contain substances that addition, Tongkat Ali Plus not induced increase in the are toxic to organisms. [18 - 20] Tongkat Ali Plus® is a mean values of chromosomal aberrations and MNPCEs[24]mixture of *Eurycoma longifolia* Jack and *Lepidium* in both sexes of rat. Li et al.studied the mutagenicity *meyenii* (Maca) powder extracts as active ingredients. In the current study *in vivo* and *in vitro* studies was conducted to evaluate the safety of Tongkat Ali Plus®.and clastogenicity of *Eurycoma longifolia* root extract. They reported that neither mutagenic nor clastogenic effects were found *in vitro* or *in vivo*. These findings The results showed that there were no positive suggested that Tongkat Ali Plus did not cause neither mutagenic responses to Tongkat Ali Plus® with any of the Base Pair Substitution

bacterial strains TA 100 and *in vitro* nor *in vivo* genotoxicity. Meanwhile, Ming et al.[23] find that *Eurycoma longifolia* extract did not alter the relative polychromatic erythrocytes, nor did not increase the frequencies of micro- nucleated polychromatic erythrocytes in mouse peripheral blood cells. In the same time, several arguments indicated that maca is safe.[25] In addition; Valerio and Gonzales[11] confirmed that maca is not mutagenic but it contains several beneficial compounds that have anticarcinogenic properties. Studies on the reproductive toxicity of treatment with Tongkat Ali Plus® are limited, so that, the current study is aimed at shedding more light on the effects of this drug on the pregnant females reproductive performance and the fetuses sired with treated parents. The results of the reproductive toxicity investigation showed that Tongkat Ali Plus® was significantly decreased the pregnant female body weights at gestation day 14 and 20 as compared to control group. This result was concordant with investigation of Solomon et al.[26] on the effects of Tongkat Ali on body and organs weights as well as functional sperm parameters in terms of safety and efficacy in the management of male infertility. They find fetuses. The fertility index and litter size of Sprague–Dawley rats treated with (TAF273) were significantly increased and the percentage of pre- and post-implantation loss and late resorption were decreased in the TAF273-treated dams. In addition, freeze- dried maca aqueous extract did not reveal any toxic effect on the normal development of pre-implanted mouse embryos. [28] Maca had been reported to have a low degree of acute oral toxicity in animals and low cellular toxicity *in vitro*.[11] Several studies on Tongkat Ali showed no toxic effects on Wistar rats in acute, sub acute, or chronic dosing periods.[22, 29]

CONCLUSION

In the present study, we evaluate the genotoxicity and the reproductive toxicity of Tongkat Ali Plus®. The results support earlier reports on the safety of the treatment with Tongkat Ali. It appears safe for possible treatment of male infertility and ageing male problems.that Tongkat Ali was significantly decreased the animal's body weight and this was attributed to the increase in serum testosterone concentration. In addition, Tongkat Ali Plus® pre-mating and during pregnancy at the three doses has not significantly changed the percentage of number of pubs, pre- and post-implantation losses, and litter size at zero days. Whereas, the high dose significantly decreased the pubs numbers and weights at 4 day of lactation when compared to control and other treated groups. Low et al.[27] showed that no toxic symptoms were observed on the quassinoid-rich *Eurycoma longifolia* extract (TAF273) treated pregnant female rats and their Taken together, this makes the use of Tongkat Ali Plus as traditional remedy a safe alternative option in treating and managing idiopathic male infertility.

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