

Acute Toxicity Studies and Comparative effect of *Kigeria Pinnata* Ethanolic leaf extract on Hepatic Functions in Albino Wistar rats treated high fat diet.

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Abstract

The aim of this study was designed to evaluate the activity of the *Kigeria pinnata* ethanolic leaf extract on liver functions in matured albino Wistar rats exposed to high fat diet. *Kigeria pinnata* leaves were obtained, air dried, powdered and extracted in a Soxhlet apparatus in 4000ml ethanol solution. Liver function indicators were assessed on rat models fed with palm oil and coconut milk. The acute toxicity test of the extract was carried out by the Lorke's method. Results showed that *Kigeria pinnata* ethanolic leaf extract significantly ($p < 0.05$), lowered plasma Alkaline phosphatase (ALP), Alanine amino transferase (ALT) and Aspartate amino transferase (AST) relative to values recorded for fat fed rats and significantly ($P < 0.05$), raised plasma Albumin (ALB) and Total protein levels compared to values recorded for fat fed rats. Results also indicated moderate change in body and organ weights in *Kigeria pinnata* treated rats relative to the control, but, significantly ($P < 0.05$), increased body and organ weights in high fat diet exposed rats, relative to values recorded for the control. Toxicity studies suggested that, the extract was safe at the dose; 2449.49mg/kg body weights of the test models. Overall findings from this study showed that the ethanolic extract exhibited no potentials for hepato-cellular injury and may possess hepato-protective properties in several ethnobotanical applications.

Keywords: Pharmacognosy, Lorke's method, Hyperlipidemia, Acute toxicity, Hepatotoxicity.

Introduction

The universal role of ethnobotanicals in ameliorating disease condition and therefore providing useful folk healthcare delivery is overwhelmingly significant in several areas of health care systems. Pharmacognosy, the scientific study of medicinal plants, therefore remains the hub of pharmacopoeial identification, modern pharmacological isolation and testing procedures that provide new plant drugs as purified substances in medicine [68]. Historically, development of potent drugs as novel compounds in orthodox medicines stems from thorough ethnopharmacological screening of popular traditional plant medicines and herbal concoctions [15]. *Kigeria pinnata*, otherwise called *Kigeria africana*, but, commonly and colloquially, known as cucumber tree, *Tember* (in Tiv speaking ethnic group of Benue state-Nigeria), or

Worsborn, is a huge ethnomedicinal plant that has found its way on the tables of ethnopharmacological laboratories for its attractive metabolomic profile [6].

Recently, *Kigeria pinnata* has been demonstrated to be great in folkore medicines [39]. Traditionally, the plant is used in the treatment of stroke, hypertension, diabetes, malaria, dysentery, skin disease, epilepsy, gynecological problems, inflammatory disorders, worm infestations, respiratory distress and ulcerative conditions [89]. Pharmacological investigations on the bark, fruit, and leaf extracts of the plant showed that *Kigeria pinnata* has profound hypolipidemic activity [89], microbial inhibition [3], anti-neoplastic potentials [49], gynecological healing properties, [26], central nervous system activity [85], and antidiabetic effects [73].

Palm oil is an ideal household cooking ingredient. It is used in commercial food and cosmetic industries in huge quantities due largely to its high oxidative stability properties [22]. Palm oil is a fatty liquid and as with all fats, it is composed of fatty acids and a glycerol back-bone held together by ester bond [21]. Palm oil as a dietary regimen has profound impact on health and disease development [21].

Coconut milk is produced from the endosperm or meat of the coconut fruit and has good role in diet with good physiological attributes [30]. Coconut milk contains approximately 20% of virgin oil and 80% of water [53]. Coconut oil consist predominantly of medium chain saturated fatty acids [32]. Coconut also contains traces of certain derivatives like betaines, polyol esters, and polyphenols like galic acid [18].

Palm oil and coconut oil therefore remain a major source of dietary lipids in many counties particularly the developing nations [18]. Critical assessment of the nutritional value of lipids point to increased pathophysiological alteration in metabolism and health [80]. Increased dietary lipids lead to a build-up of abnormal serum lipid concentrations in blood; a condition called dyslipidemia. Dyslipidemia has been established to be a predisposing factor for cardiovascular complications and induced liver dysfunction [90] [14].

The aminotransferases are enzymes involved in the transfer of an amino group from an α -amino acid to an α -oxoacid requiring pyridoxal phosphate cofactor for optimal activity [24]. Two common aminotransferases of clinical significance are the aspartate aminotransferase (AST), and alanine aminotransferases (ALT). Aspartate aminotransferase and alanine aminotransferase are located predominantly in the cells of the skeletal muscle (myocytes), liver (Hepatocytes), kidney and the heart. ALT is found exclusively in the cytoplasm of these cells and AST is distributed in the mitochondria and cytoplasm of these cells [27]. The appearance and presence of these two aminotransferases in blood is of serious diagnostic importance. It has been demonstrated that concentrations of these enzymes in plasma are indicative of tissue or cellular damage.

Alkaline phosphatase (ALP), belongs to a group of enzymes that catalyzes the breakdown of organic phosphates due to high PH. ALP is commonly activated by bivalent ions such as Mg^{2+} and Mn^{2+} but, easily inhibited by borate, phosphate and cyanide [1]. Plasma elevation of ALP is typically diagnostic of hepatobiliary and bone disorders. In all forms of hepatobiliary diseases, plasma levels are higher in biliary obstruction than hepatocellular damage.

Total plasma proteins are most abundant organic molecules in the serum. Major serum proteins of diagnostic significance that have been demonstrated include albumin, globulins and prothrombin [51]. Albumin is associated with the maintenance of blood volume and blood pressure; globulins (antibodies) are immune polypeptides associated with body defenses and prothrombin proteins are for platelet aggregation during

clot-formation. Plasma proteins are synthesized essentially by the liver. Studies have shown that induced hepatocellular damage results in marked decreases in the concentrations of total plasma protein [25]. Albumin is synthesized by the parenchymal cells of the liver with the synthetic rate regulated by dietary protein and colloidal osmotic pressure (COP) [96]. Albumin binds and transports in blood a wide variety of molecules which include; fatty acids, phospholipids, hormones, amino acids, drugs, bilirubin and metallic ions [23].

Aim and Objectives of the Study

- i. To establish the lethal dose (LD₅₀) of *Kigeria pinnata* ethanolic leaf extract.
- ii. To determine the comparative effect of *Kigeria pinnata* ethanolic leaf extract on liver functions in rats orally administered palm oil and coconut milk.
- iii. To determine the comparative effect of *Kigeria pinnata* ethanolic leaf extract on change in body weight and estimation of organ weights of rats orally administered palm oil and coconut milk

Materials and methods

Collection and Identification of Plant Sample

Kigelia pinnata leaves were obtained in the month of August, 2015 from Kumbur *Aga* forest Mbakume, in Gwer-East Local Government Area of Benue State. The plant leaves were identified and authenticated by a Botanist in the Department of Botany, University of Agriculture Makurdi, Benue State.

Preparation of plant extract and oil source

The *Kigelia pinnata* leaves obtained from Kumbur *Aga* forest were properly washed and rinsed with tap water to remove the debris. The leaves were air-dried for two weeks in the Department of Biochemistry, University of Calabar, - Calabar, - Nigeria. The dried leaves were then ground to powder using mechanical grinder. About 250g of the dried leaf powder was extracted using the soxhlet apparatus, concentrated by evaporation and stored in the refrigerator for use. Fresh palm oil and coconut fruits were obtained from Akim Market in Calabar. The oil was also stored in the refrigerator for use, while coconut fruits were opened, the meat removed and grated. The grated meat was squeeze and filtered using Muslim cloth to obtain the coconut milk and also stored in the refrigerator for use.

Animal Handling and Treatment.

Forty-Eight (48) male albino Wistar rats weighting between 120-160grams, were obtained from the Animal farm of the Department of Biochemistry, University of Calabar, Calabar, Nigeria. The rats were kept in the animal house of the Department of Biochemistry, University of Calabar. Rats were then distributed randomly into eighty (6) groups with six animals per group and acclimatized for two (2) weeks under an experimental condition of 12 hours light -dark cycle and prevailing ambient temperature of 25°C. The animals were fed with the normal rat chow and allowed access to tap water *ad libitum*.

Experimental design and animal groups distribution

Table 1: Distribution of Rats into Experiment groups

Group	Number	Treatment
1	6	Normal rat chow (Control) (CO)
2	6	0.49g/kg body weight KP
3	6	5ml/kg body weight PO
4	6	5ml/kg body weight CM
5	6	2.5ml/kg body weight each PO and CM
6	6	0.25g/kg body weight of KP and 2.5m/kg body weight each of PO and CM (after 2 hours of KP administration).

Treatment with the respective test substances (KP, PO, CM and CO) was done orally

using intragastric cannula per day. All treatments lasted for 30 days. KP

(*Kigelia pinnata*) PO (Palm Oil) CM (Coconut Milk) CO (Control).

Collection and preparation of blood sample for serum lipids profile analyses.

Blood samples were obtained from rats by cardiac puncture under chloroform vapour anaesthesia, 24-hours after the last day of experimental treatment. Blood samples for analyses were collected into sterile, plain bottles.

The blood sample collected for analyses were spinned in a centrifuge at 3000rpm for 10 minutes

to obtain the serum. Serum was collected in dry sample containers stored in a refrigerator and used for serum...

Acute Toxicity (LD₅₀) determination of *Kigelia pinnata* leaf extract.

The LD₅₀ was carried out using the Lorke's method (1993). It involved a total number of thirteen (13) male mice. The test was carried out in phases. Phase one employed a total of nine (9) mice, grouped into three (3), i.e. three mice per group. Group one received 500mg/kg of the extract, group two received 1000mg/kg of the extract and group three received 2000mg/kg of the extract. All administrations were done by intra-peritoneal route. The animals were monitored for four (4) hours, then intermittently for the next 6 hours and over a period of 24-hours. The numbers of dead animals were noted. From the result of phase one, the second phase (II) was carried out. In phase II a total of four Mice were used and group into four groups, one mice per group. Group one received 3000mg/kg, group received 4000mg/kg and group three received 5000mg/kg of the extract, and group four received 1 ml of tween 80.

The animals were then monitored for any death. The LD₅₀ was then calculated using the relationship.

$$Ld_{50} = \sqrt{DoxD} \quad (1)$$

D₀=Highest dose that gave no mortality

D₁₀₀=Lowest dose that produced mortality.

Statistical Analysis

Data are presented as mean $\pm$ Standard error of mean (SEM). Results were analyzed using one-way analysis of variance (ANOVA) with SPSS window. Tukey post hoc test used for pairwise comparison, and differences were considered significant at $P < 0.05$ (5% level of confidence limit). The normal control group was compared with the fat-diet fed group and all other treatment groups were compared with the fat-diet fed group.

Results

Acute Toxicity Test (LD_{50}) for ethanolic leaf extracts of *Kigelia pinnata* Plant by Lorke's method.

Table 2: The Results of acute toxicity test of *Kigelia pinnata* extract on rats.

Phase	Doses (mg/kg)	Number of Death
1	500	0/3
	1000	0/3
	2000	0/3
2	3000	0/1
	4000	1/1
	5000	1/1
Contro	tween 80	0/1

$$(Ld_{50}) = \sqrt{3000 \times 4000}$$

$$= 3464.10 \text{ mg/kg.}$$

Effect of *Kigelia pinnata* leaf extract on serum liver function indicators

Table 3: Effect of *Kigelia pinnata* ethanolic leaf extract on serum liver function indicators.

Groups	AST (U/L)	ALT (u/L)	ALP (u/L)	TP (g/dL)	ALB (g/dL)
Group 1	20.27 $\pm$ 0.49	28.42 $\pm$ 0.33	48.84 $\pm$ 0.65	3.20 $\pm$ 0.04	2.31 $\pm$ 0.07
Group 2	20.88 $\pm$ 0.45	28.24 $\pm$ 0.35	51.84 $\pm$ 0.50*	3.08 $\pm$ 0.05	2.28 $\pm$ 0.02
Group 3	36.12 $\pm$ 0.52*#	38.58 $\pm$ 0.24*#	62.95 $\pm$ 0.63*#	2.53 $\pm$ 0.05*#	1.35 $\pm$ 0.03*#
Group 4	24.00 $\pm$ 0.41*# ϕ	28.21 $\pm$ 0.61 ϕ	51.52 $\pm$ 0.31* ϕ	3.00 $\pm$ 0.09 ϕ	2.23 $\pm$ 0.05 ϕ
Group 5	32.86 $\pm$ 0.60*# ϕ †	37.28 $\pm$ 0.50*#†	60.39 $\pm$ 0.37*#†	2.70 $\pm$ 0.04*#†	1.73 $\pm$ 0.04*# ϕ †
Group 6	22.27 $\pm$ 0.31 ϕ †	28.17 $\pm$ 0.59 ϕ †	50.67 $\pm$ 0.82 ϕ †	2.93 $\pm$ 0.05* ϕ	2.16 $\pm$ 0.04 ϕ †

Values presented Mean $\pm$ SEM. n = 4. * = significantly different to control at $P < 0.05$, # = significantly, relative to Group 2 after treatment at $P < 0.05$ ϕ = significantly, relative to Group 3 at $P < 0.05$, † = significant relative to Group 4 at $P < 0.05$, ‡ = significant relative to Group 5 at $P < 0.05$

Table 4: Change in body weights of groups in 30days.

Group	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)	Week 5 (g)
Control	134.75	147.13	159.40	168.70	180.63
	±0.61	±0.95	±0.88	±0.09	±0.68
II	153.63	166.13	172.90	187.08	196.35
	±0.57	±0.74	±0.84	±0.95	±0.52
III	141.90	166.55	179.43	187.30	210.13
	±1.37	±0.59	±1.35	±1.21	±0.09
IV	142.33	160.05	171.00	180.73	191.29
	±1.23	±0.97	±1.28	±0.56	0.85
V	123.80	136.75	137.65	140.05	165.30
	±1.10	±1.60	±1.65	±1.64	±0.14
VI	159.87	159.53	161.13	173.40	189.67
	±1.26	±1.32	±0.90	±1.48	±0.82

Value are expressed as mean ± SEM, n=4

Table 5: Liver and Kidney weights of rats on sacrificed

Group	Kidney weight (g)	Liver weight (g)
Control	1.15	6.90
	±0.09	±0.41
II	1.33	6.70
	±0.15	±0.66
III	1.25	7.08
	±0.09	±0.46
IV	1.23	6.43
	±0.10	±0.38
V	1.00	6.60
	±0.05	±0.30
VI	1.10	6.60
	±0.14	±0.64

Value are expressed as mean ± SEM, n=4

Discussion

The result of the acute toxicity test of *Kigeria pinnata* leaf extracts indicates that, the extract did not produce any noticeable toxicity in the test models up to dose level of 3000mg/kg body weight of rats even after 48hours, but produced toxic symptoms of restlessness, loss of appetite and motility at the dose level of

4000mg/kg body weight and dose level 5000mg/kg body weight. The *Kigeria pinnata* leaf extract therefore has high lethal dose and could be safe for therapeutic purposes. This result partly agrees with the report of [9], which classified *Kigeria pinnata* plant as a non-toxic medicinal plant but, expressed the safe dose of 4000mg/kg body weight, in contrast, to this result that states the safe dose of *Kigeria pinnata* plant as 3000mg/kg body weight.

The functional integrity of the liver tissue can be examined from the assay of some serum enzyme activity and serum protein levels. The activities of serum enzymes such as ALT, AST, GGT, ALP and serum protein levels, are of huge diagnostic significance. Increased serum ALT, AST and ALP activity is diagnostic for hepato-cellular injury and bone abnormality []. Fatty infiltrations of the liver in hyperlipidemias have been demonstrated to be critical with increased serum ALT, AST and ALP levels [7]. Hyperlipidemia has been reported to simulate increased synthesis and secretion of hepatic metabolic enzymes leading to elevated serum ALT, AST, and ALP [4]. [], demonstrated that, serum primary biliary cirrhosis (PBC) and non-alcoholic fatty liver disease leads to massive leakage of ALP and ALT in blood.

Results of this study reported no observed changes in serum AST, ALT, ALP, albumin and total protein levels in rats exposed to *Kigeria pinnata* extract and co-administered with palm oil and coconut milk relative to the control group. Results also showed no noticeable change in AST, AST, ALP, albumin and total protein levels in rats exposed to coconut milk only. Results however, indicated elevated ALT, AST and ALP activity, but, decreased albumin and total protein levels in rats, treated with high amount of palm oil. The findings are in consonance with the report of [29], which stated that coconut and palm oil have a dose-dependent effect on liver enzymes such as ALT, AST and ALP. The result of the study agrees with the works of [55], and [6], demonstrating *Kigeria pinnata* extract to contain potent antioxidants, hypolipidemic and anti-inflammatory activity to liver and kidney tissues. The results also agree with the report on beneficial action of virgin coconut oil on lipid fractions and in-vitro LDL oxidation [71], that, coconut oil enhances protein synthesis by the liver hepatocytes.

The possible mechanism of action of *Kigeria pinnata* extract, stems from its huge content of potent antioxidants. Polyphenols and flavonoids contained in *Kigeria pinnata* leaf extracts have been demonstrated to activate adenosine monophosphate kinase and the phosphorylation of acetylcoA carboxylase. Once phosphorylated, acetylcoA carboxylase activity becomes inhibited, thereby, preventing fatty acid synthesis, ameliorating avenue for fatty liver [99].

Conclusion

It may be concluded from the results of this study that; oral exposure to *Kigeria pinnata* leaf extracts contains some active principles that may provide protection against lipid-induced tissue injury, as indicated in proportionately, normal serum liver enzyme activities and protein amount. It may also be concluded from the findings to the study that, *Kigeria pinnata* extract has no adverse effect on liver and kidney tissues, as indicated in the remodeling of the weights of these organs. Consumption of palm oil beyond certain amounts may lead to obesity thereby predisposing the individual to metabolic crisis.

					95% Confidence Interval for Mean		Minimu m	Maximu m
					Lower Bound	Upper Bound		
	N	Mean	Std. Deviation	Std. Error				



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AST	Contro 1	4	20.2675	.98632	.49316	18.6980	21.8370	19.11	21.51
	G2	4	20.8750	.89213	.44607	19.4554	22.2946	19.96	21.94
	G3	4	36.1225	1.03744	.51872	34.4717	37.7733	35.20	37.52
	G4	4	23.9975	.81299	.40650	22.7038	25.2912	23.10	24.73
	G5	4	32.8600	1.19474	.59737	30.9589	34.7611	31.66	34.41
	G6	4	22.2700	.62594	.31297	21.2740	23.2660	21.61	23.10
	Total	24	26.0654	6.33202	1.29252	23.3916	28.7392	19.11	37.52
ALT	Contro 1	4	28.4200	.66353	.33176	27.3642	29.4758	27.52	29.10
	G2	4	28.2350	.70774	.35387	27.1088	29.3612	27.56	29.22
	G3	4	38.5825	.48114	.24057	37.8169	39.3481	37.94	39.10
	G4	4	28.2125	1.21088	.60544	26.2857	30.1393	27.02	29.40
	G5	4	37.2800	1.00013	.50007	35.6886	38.8714	36.30	38.62
	G6	4	28.1700	1.17627	.58813	26.2983	30.0417	26.80	29.20
	Total	24	31.4833	4.74349	.96826	29.4803	33.4863	26.80	39.10
ALP	Contro 1	4	48.8425	1.30303	.65152	46.7691	50.9159	47.26	49.96
	G2	4	51.8350	1.00351	.50176	50.2382	53.4318	50.88	53.10
	G3	4	62.9450	1.26147	.63073	60.9377	64.9523	61.44	64.44
	G4	4	51.5200	.61995	.30997	50.5335	52.5065	50.84	52.23
	G5	4	60.3925	.73898	.36949	59.2166	61.5684	59.86	61.48
	G6	4	50.6700	1.64104	.82052	48.0587	53.2813	49.25	53.03
	Total	24	54.3675	5.50940	1.12460	52.0411	56.6939	47.26	64.44
TP	Contro 1	4	3.2000	.08165	.04082	3.0701	3.3299	3.10	3.30
	G2	4	3.0750	.09574	.04787	2.9227	3.2273	3.00	3.20
	G3	4	2.5250	.09574	.04787	2.3727	2.6773	2.40	2.60
	G4	4	3.0000	.18257	.09129	2.7095	3.2905	2.80	3.20
	G5	4	2.7000	.08165	.04082	2.5701	2.8299	2.60	2.80
	G6	4	2.9250	.09574	.04787	2.7727	3.0773	2.80	3.00
	Total	24	24.4250	.63690	.25500	23.2444	24.9439	23.50	24.50



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Total	24	2.9042	.25277	.05160	2.7974	3.0109	2.40	3.30
ALB Contro 1	4	2.3125	.14408	.07204	2.0832	2.5418	2.10	2.42
G2	4	2.2775	.03304	.01652	2.2249	2.3301	2.24	2.31
G3	4	1.3500	.05099	.02550	1.2689	1.4311	1.28	1.40
G4	4	2.2275	.09845	.04922	2.0709	2.3841	2.10	2.31
G5	4	1.7250	.08103	.04052	1.5961	1.8539	1.65	1.84
G6	4	2.1575	.08732	.04366	2.0186	2.2964	2.04	2.25
Total	24	2.0083	.36953	.07543	1.8523	2.1644	1.28	2.42

Dependent Variable	(I) GROUPS1	(J) GROUPS1	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval
						Lower Bound
AST	control	G2	-.60750	.66616	.938	-2.7246
		G3	-15.85500*	.66616	.000	-17.9721
		G4	-3.73000*	.66616	.000	-5.8471
		G5	-12.59250*	.66616	.000	-14.7096
		G6	-2.00250	.66616	.070	-4.1196
	G2	control	.60750	.66616	.938	-1.5096
		G3	-15.24750*	.66616	.000	-17.3646
		G4	-3.12250*	.66616	.002	-5.2396
		G5	-11.98500*	.66616	.000	-14.1021
		G6	-1.39500	.66616	.333	-3.5121
	G3	control	15.85500*	.66616	.000	13.7379
		G2	15.24750*	.66616	.000	13.1304
		G4	12.12500*	.66616	.000	10.0079
		G5	3.26250*	.66616	.001	1.1454
		G6	13.85250*	.66616	.000	11.7354
	G4	control	3.73000*	.66616	.000	1.6129



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		G2	3.12250*	.66616	.002	1.0054
		G3	-12.12500*	.66616	.000	-14.2421
		G5	-8.86250*	.66616	.000	-10.9796
		G6	1.72750	.66616	.150	-.3896
	G5	control	12.59250*	.66616	.000	10.4754
		G2	11.98500*	.66616	.000	9.8679
		G3	-3.26250*	.66616	.001	-5.3796
		G4	8.86250*	.66616	.000	6.7454
		G6	10.59000*	.66616	.000	8.4729
	G6	control	2.00250	.66616	.070	-.1146
		G2	1.39500	.66616	.333	-.7221
		G3	-13.85250*	.66616	.000	-15.9696
		G4	-1.72750	.66616	.150	-3.8446
		G5	-10.59000*	.66616	.000	-12.7071
ALT	control	G2	.18500	.64696	1.000	-1.8711
		G3	-10.16250*	.64696	.000	-12.2186
		G4	.20750	.64696	.999	-1.8486
		G5	-8.86000*	.64696	.000	-10.9161
		G6	.25000	.64696	.999	-1.8061
	G2	control	-.18500	.64696	1.000	-2.2411
		G3	-10.34750*	.64696	.000	-12.4036
		G4	.02250	.64696	1.000	-2.0336
		G5	-9.04500*	.64696	.000	-11.1011
		G6	.06500	.64696	1.000	-1.9911
	G3	control	10.16250*	.64696	.000	8.1064
		G2	10.34750*	.64696	.000	8.2914
		G4	10.37000*	.64696	.000	8.3139
		G5	1.30250	.64696	.373	-.7536
		G6	10.41250*	.64696	.000	8.3564
	G4	control	-.20750	.64696	.999	-2.2636





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			G2	-.02250	.64696	1.000	-2.0786
			G3	-10.37000*	.64696	.000	-12.4261
			G5	-9.06750*	.64696	.000	-11.1236
			G6	.04250	.64696	1.000	-2.0136
			G5 control	8.86000*	.64696	.000	6.8039
			G2	9.04500*	.64696	.000	6.9889
			G3	-1.30250	.64696	.373	-3.3586
			G4	9.06750*	.64696	.000	7.0114
			G6	9.11000*	.64696	.000	7.0539
			G6 control	-.25000	.64696	.999	-2.3061
			G2	-.06500	.64696	1.000	-2.1211
			G3	-10.41250*	.64696	.000	-12.4686
			G4	-.04250	.64696	1.000	-2.0986
			G5	-9.11000*	.64696	.000	-11.1661
		ALP	G2 control	-2.99250*	.81239	.018	-5.5743
			G3	-14.10250*	.81239	.000	-16.6843
			G4	-2.67750*	.81239	.040	-5.2593
			G5	-11.55000*	.81239	.000	-14.1318
			G6	-1.82750	.81239	.264	-4.4093
			G2 control	2.99250*	.81239	.018	.4107
			G3	-11.11000*	.81239	.000	-13.6918
			G4	.31500	.81239	.999	-2.2668
			G5	-8.55750*	.81239	.000	-11.1393
			G6	1.16500	.81239	.707	-1.4168
			G3 control	14.10250*	.81239	.000	11.5207
			G2	11.11000*	.81239	.000	8.5282
			G4	11.42500*	.81239	.000	8.8432
			G5	2.55250	.81239	.054	-.0293
			G6	12.27500*	.81239	.000	9.6932
			G4 control	2.67750*	.81239	.040	.0957





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		G2	-.31500	.81239	.999	-2.8968
		G3	-11.42500*	.81239	.000	-14.0068
		G5	-8.87250*	.81239	.000	-11.4543
		G6	.85000	.81239	.896	-1.7318
		G5 control	11.55000*	.81239	.000	8.9682
		G2	8.55750*	.81239	.000	5.9757
		G3	-2.55250	.81239	.054	-5.1343
		G4	8.87250*	.81239	.000	6.2907
		G6	9.72250*	.81239	.000	7.1407
		G6 control	1.82750	.81239	.264	-.7543
		G2	-1.16500	.81239	.707	-3.7468
		G3	-12.27500*	.81239	.000	-14.8568
		G4	-.85000	.81239	.896	-3.4318
		G5	-9.72250*	.81239	.000	-12.3043
TP	control	G2	.12500	.07862	.615	-.1248
		G3	.67500*	.07862	.000	.4252
		G4	.20000	.07862	.163	-.0498
		G5	.50000*	.07862	.000	.2502
		G6	.27500*	.07862	.026	.0252
	G2 control	G2	-.12500	.07862	.615	-.3748
		G3	.55000*	.07862	.000	.3002
		G4	.07500	.07862	.926	-.1748
		G5	.37500*	.07862	.002	.1252
		G6	.15000	.07862	.429	-.0998
	G3 control	G3	-.67500*	.07862	.000	-.9248
		G2	-.55000*	.07862	.000	-.7998
		G4	-.47500*	.07862	.000	-.7248
		G5	-.17500	.07862	.274	-.4248
		G6	-.40000*	.07862	.001	-.6498
	G4 control	G4	-.20000	.07862	.163	-.4498





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		G2	-.07500	.07862	.926	-.3248
		G3	.47500*	.07862	.000	.2252
		G5	.30000*	.07862	.014	.0502
		G6	.07500	.07862	.926	-.1748
		G5 control	-.50000*	.07862	.000	-.7498
		G2	-.37500*	.07862	.002	-.6248
		G3	.17500	.07862	.274	-.0748
		G4	-.30000*	.07862	.014	-.5498
		G6	-.22500	.07862	.092	-.4748
		G6 control	-.27500*	.07862	.026	-.5248
		G2	-.15000	.07862	.429	-.3998
		G3	.40000*	.07862	.001	.1502
		G4	-.07500	.07862	.926	-.3248
		G5	.22500	.07862	.092	-.0248
		ALB control	G2	.03500	.06346	.993
		G3	.96250*	.06346	.000	.7608
		G4	.08500	.06346	.760	-.1167
		G5	.58750*	.06346	.000	.3858
		G6	.15500	.06346	.194	-.0467
		G2 control	-.03500	.06346	.993	-.2367
	G3	.92750*	.06346	.000	.7258	
	G4	.05000	.06346	.966	-.1517	
	G5	.55250*	.06346	.000	.3508	
	G6	.12000	.06346	.438	-.0817	
	G3 control	-.96250*	.06346	.000	-1.1642	
	G2	-.92750*	.06346	.000	-1.1292	
	G4	-.87750*	.06346	.000	-1.0792	
	G5	-.37500*	.06346	.000	-.5767	
	G6	-.80750*	.06346	.000	-1.0092	
	G4 control	-.08500	.06346	.760	-.2867	



	G2	-.05000	.06346	.966	-.2517
	G3	.87750*	.06346	.000	.6758
	G5	.50250*	.06346	.000	.3008
	G6	.07000	.06346	.874	-.1317
G5	control	-.58750*	.06346	.000	-.7892
	G2	-.55250*	.06346	.000	-.7542
	G3	.37500*	.06346	.000	.1733
	G4	-.50250*	.06346	.000	-.7042
	G6	-.43250*	.06346	.000	-.6342
G6	control	-.15500	.06346	.194	-.3567
	G2	-.12000	.06346	.438	-.3217
	G3	.80750*	.06346	.000	.6058
	G4	-.07000	.06346	.874	-.2717
	G5	.43250*	.06346	.000	.2308

*. The mean difference is significant at the 0.05 level.

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