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Effects of chronic administration of ethanolic extract of Kolanut *(Cola nitida)* and caffeine on vascular function

HM Salahdeen[1], AO Omoaghe[1], GO Isehunwa[2], BA Murtala[1] and ARA Alada[2]

Department of Physiology, College of Medicine, Lagos State University, Ikeja, Lagos and Department of Physiology, University of Ibadan, Ibadan, Nigeria

Abstract

Background: Kolanut (*Cola nitida*) is consumed in virtually every part of the world. The caffeine content of kolanut is scarce and the number of investigations studying the health benefits of kolanut is negligible compared to coffee.

Objective: The present study was designed to identify the caffeine content of kolanut and evaluate the effect of its chronic consumption on cardiovascular functions in rats.

Methods: The caffeine content of kolanut was determined by Gas chromatography-mass spectrometry (GC-MS). Wistar albino rats were divided into four groups (10 Rats/group). Kolanut extract (11.9 mg/kg), caffeine extracted from kolanut (7.5 mg/kg), decaffeinated of kolanut extract (6 mg/kg) and distilled water (control) was administered orally to each group for six-weeks. Effect of treatment on body weight, blood pressure and relaxation response to acetylcholine (ACh) and sodium nitroprusside (SNP) of the aortic rings was assessed.

Results: The total caffeine content of kolanut extract was found to be 51% and it was 96% pure from GC-MS analysis. Chronic consumption of kolanut and caffeine significantly ($p<0.05$) decreased body weight. Similarly, kolanut extract decaffeinated kolanut and caffeine significantly ($p < 0.05$) reduced the contractile response to noradrenaline and higher potassium solution. Kolanut extract and caffeine also significantly ($p < 0.05$) increased the mean arterial blood pressure. Caffeine and kolanut consumption reduced the relaxation response to both acetylcholine and sodium nitroprusside. Atropine and L-NAME considerably inhibit the ACh-induced relaxation of the rat aortic ring suggesting the involvement of cholinergic mechanism. However, indomethacin (10^{-4}M) also attenuated the ACh response indicating involvement of protanoids.

Conclusion: The results suggest that treatment with both kolanut extract and caffeine had similar characteristics between the two groups with no significant differences in the ACh-induced relaxation of the ring suggesting that the action of kolanut extract is due to its caffeine content.

Keywords: *Kolanut extract, caffeine- induced relaxation, aortic rings, decaffeinated caffeine.*

Correspondence: Dr. H.M. Salahdeen, Department of Physiology, Smooth Muscles Research Group, Lagos State University, College of Medicine, Ikeja, Lagos, Nigeria. E-mail: hmsalahdeen@yahoo.ca; hussein.salahdeen@lasucom.edu.ng

Résumé

Introduction: La noix de cola (*Cola nitida*) est consommée dans pratiquement toutes les parties du monde. La teneur en caféine de Kola est rare et le nombre d'enquêtes qui étudient les bienfaits pour la santé de Kola est négligeable par rapport à café. La présente étude visait à déterminer la teneur en caféine du colatier et évaluer l'effet de sa consommation chronique sur les fonctions cardiovasculaires chez les rats.

Méthodes: La teneur en caféine de Kola a été déterminée par chromatographie en phase gazeuse-spectrométrie de masse (GC-MS). Les rats albinos Wistar ont été divisés en quatre groupes (10 rats / groupe). L'extrait de noix de cola (11,9 mg / kg), de la caféine extraite de noix de cola (7,5 mg / kg), de l'extrait décaféiné de kolanut (6 mg / kg) et d'eau distillée (témoins) a été administré par voie orale à chaque groupe de six semaines. Effet du traitement sur le poids corporel, la pression artérielle et relaxation réponse à l'acétylcholine (ACh), et nitroprussiate de sodium (SNP) de la crosse aortique anneaux était évaluée.

Résultats: La teneur totale en caféine de l'extrait de noix de cola a été constatée à 51% et elle est de 96% pure à partir de l'analyse GC-MS. Une consommation chronique de Kola et de la caféine de façon significative ($p <0,05$) diminution du poids corporel. Une consommation chronique de Kola et de la caféine ($p <0,05$) diminution du poids corporel. De même, l'extrait de noix de cola décaféiné kolanut et la caféine ($p <0,05$) réduit la réponse contractile à la noradrénaline et de la solution de potassium supérieur. Extrait de noix de cola et de la caféine a également à ($p <0,05$) ont augmenté la pression artérielle moyenne. La consommation de caféine et colatier réduit la réponse de relaxation à l'acétylcholine et sodium nitroprusside Atropine et le L-NOM inhibent considérablement la relaxation ACh-incitée du rat l'anneau aortique suggérant le rôle de mécanisme cholinergique. Cependant, l'indométacine (10-4M) a également atténué la réponse ACh indiquant l'implication de protanoides.

Conclusion: Les résultats suggèrent que le traitement combiné d'extrait de noix de cola et de la caféine avait des caractéristiques similaires entre les deux groupes sans différence significative dans la relaxation induite par l'ACh sur l'anneau ce qui suggère que l'action de l'extrait de noix de cola est due à sa teneur en caféine.

displacement by the tissue. Each of the rings was under a passive tension of 2 g and superfused in a 50 mL double-jacketed organ bath with PSS at 37 °C and gassed with 95% O_2: 5% CO_2 mixture. The pH of the PSS was usually between 7.35-7.40, and all baths used simultaneously had a parallel connection to the source of the PSS. The composition of PSS was (mM): NaCl, 118.0; KCl, 4.7; $NaHCO_3$, 15.0; $MgSO_4$, 1.2; $CaCl_2.2H_2O$, 1.6; KH_2PO_4 1.2 and glucose, 11.5. The mounted ring preparations were subsequently left to equilibrate for 90 min during which they were challenged with 10^{-7}M noradrenaline at 30 min interval.

Experimental protocol

The stabilization was required to ensure a consistent response of the aortic rings throughout the experiment. At the end of the 90 min stabilization period, the relaxation response study to ACh and SNP was carried out. The relaxation response to ACh was assessed in endothelial intact aortic rings. Aortic rings were precontracted with 10^{-7} M noradrenaline, and after the contraction had reached a plateau, cumulative doses of ACh (10^{-9}–10^{-5} M) and SNP (10^{-10}–10^{-5} M) were added to the organ bath.

Since residual caffeine molecules may remain in the preparation obtained from treated animals, all experiments were performed in the presence of atropine (10^{-4}M), COX and NOS product involvement in the contractile effects of noradrenaline were evaluated by comparing the effect of Ach on rings pre-incubated with indomethacin (10^{-5} M) L-NAME (10^{-4}M) or indomethacin (10^{-5} M) plus L-NAME (10^{-4}M) for 15 min with those observed in untreated rings.

Statistical analysis

Data are expressed as means ± SE, where n equals the number of animals from which blood vessels were isolated. The data were analyzed using one-way ANOVA. The Student-Newman-Keuls post hoc test was used to identify differences between individual means. The confidence interval was set at 95%, so that in all cases, results with a value of $p< 0.05$ were considered statistically significant.

Results

In the GC-MS analysis of crude ethanolic kolanut extract, a total of 39 compounds were identified. The predominant compounds identified was caffeine with retention time of (RT: 19.65mins; caffeine content is 50.3% with 96.5% pure) (data not shown); 9, 12 - Octadecadienoic acid, ethyl ester RT: 22.353 of 8.636% quality 99; Hexadecanoic acid, ethyl ester RT: 20.43 of 7.968% and quality 99; Methyl 2-octylcyclopropene-1-octanoate RT: 22.099 of 7.02 91 in quality; 9-Octadecenoic acid, ethyl ester, Ethyl Oleate RT: 22.422 of 4.91% and quality 99; and Cyclohexanone, 2-methyl-5-(1-methylethenyl) Octadec-9-enoic acid Decanoic acid 10-(2-hexylcyclopropyl) RT: 23.148 of 3.794 and quality 83 (Table 1).

Table 1: GC-MS analysis of ethanolic kolanut extracts showing retention time, total percentage and identified compounds

S/N	Retention Time (min)	% Compound	Identified compound
1	19.583	36.551	Caffeine
2	20.07	46.14	Caffeine
3	21.638	1.362	9- Octadecenoic acid (Z), methyl ester
4	26.37	9.41	Diisooctyl ester
5	32.996	2.791	Octasiloxane -1-15-hexadecametyl
	Total	96.254	

Twelve major compounds were identified in GC – MS analysis of decaffeinated kolanut extract. The percentage composition of caffeine had decreased to less than five- percentage.

Body weight of Animals

At the end of the 6 week experimental period the final body weights of the rats were determined in all the groups. Table 1 shows the percentage weight gain in each group. In all groups, there was an increase in body weight after the 6 weeks period of the experiment. However, the percentage increase in weight in kolanut extract and caffeine treated animals were significantly lower ($p < 0.05$) when compared with the control. There were no significant changes in the percentage weight gain and final body weight of both the normal and decaffeinated kolanut extract when compared with their corresponding groups.

Blood pressure

At the end of the 6week experimental period, the mean arterial blood pressure (MABP) (Table 2) showed a significant ($p < 0.05$) increase in crude ethanolic kolanut extract and caffeine from kolanut groups when compared with control. However, there were no significant differences in MABP of both kolanut extract and caffeine groups. Also, MABP was significantly lower ($p < 0.05$) in the decaffeinated group when compared with control.

the methylene chloride was evaporated in the hood on a warm hot plate to isolate caffeine and the melting point of the caffeine recovered was determined [23,24].

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using a Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with a flame ionization detector and a Hewlett Packard 7683 series injectors, MS transfer line temperature of 250°C. The GC was equipped with a fused silica capillary column- HP-5MS (30 x 0.25 mm), film thickness 1.0 µm. The oven temperature was held at 50°C for 5 min holding times and raised from 50 to 250°C at a rate of 2°C /min, employing helium gas (99.999%) as a carrier gas at a constant flow rate of 22 cm/s. 1.0 mg/mL of extract (1 mg dissolved in 1 mL absolute alcohol), at a split ratio of 1:30 was injected. MS analysis was carried out on Agilent Technology Network Mass Spectrometer (Model 5973 series) coupled to Hewlett Packard Gas Chromatograph (Model 6890 series) equipped with NIST08 Library software database. Mass spectra were taken at 70 eV/200°C; a scanning rate of 1 scan/s. Identification of compounds was conducted using the database of NIST08 Library. The mass spectrum of the individual unknown compound was compared with the known compounds stored in the software database Library.

Animals

Healthy, young adult, Wistar albino male rats, weighing 200-230 g were obtained from the Animal House of the Lagos State University College of Medicine, Ikeja, Lagos State, Nigeria. The rats were fed with standard rat chow (Livestock Feeds, Ikeja, and Lagos State, Nigeria) and water *ad libitum*. The animals were maintained at standard laboratory conditions (12/12 h dark/light cycle, 20 ± 2 °C temperatures, and 65 ± 5 % humidity). The rats were fasted 12-16 h before the beginning of the experiment.

Experimental design

The rats were divided into 4 groups of 10 rats each.
Group I served as the control and was given normal saline (NS).
Group II received 11.9 mg/kg of de-caffeinated kolanut (DECAF).
Groups III was treated with 7.5 mg/kg of ethanolic extract kolanut (EEKNT).
Group IV was treated with 6 mg/kg of isolated caffeine (CAFF).
Each group received treatment orally for six-weeks. The doses (11.9 mg/kg, 7.5 mg/kg, 6 mg/kg) per body weight of decaffeinated, ethanolic kolanut extract, and caffeine used in this study, is equivalent to three cups of coffee per day in human when the conversion is based on the metabolic rate at 70kg body weight [25,26] which is based on our previous study [27].

At the end of 6-week experimental period, 5 randomly selected animals from each group were used for invasive blood pressure measurement and terminal arterial blood pressure was determined via femoral artery cannulation [27].

Determination of body weight:

The animals were weighed before and at weekly intervals throughout the period of the experiment using a Duet toploading scale (Salter Brecknell, Salter, UK). After the experimental period, the percentage weight gain was calculated. The final weights of the animals were compared across groups

Measurement of blood pressure

Invasive blood pressure measurement was carried out via arterial cannulation [27]. The rats were anaesthetized with a solution of 25% (w/v) urethane and 1% (w/v) α-chloralose injected intra-peritoneal at a dose of 5 ml/kg body weight. The anaesthetized rat was placed on its back on the operating table, the limbs were fastened to the table, and the trachea was exposed and cannulated. The blood pressure measurements were obtained by the cannulation of one femoral artery. A polyethylene cannula filled with 1% heparinised saline was inserted into the artery, tied in place, and connected via a pressure transducer model 7082 coupled to Data Capsule Model 17400 of Ugo Basile Italy.

Tissue preparation

The rats were anaesthetized with urethane and the thoracic cage was opened and heparin injected into the ventricle to prevent blood clotting. The aorta was cut at the visible ends, excised and quickly placed in a Petri dish containing cold Krebs-Henseleit physiological solution (KHS). The aorta was then freed of connective tissue and cut into 2-3 cm ring segments. Special care was taken to avoid contact with the endothelial surface during the removal and mounting of the rings. Each ring was then mounted horizontally between two fine stainless steel rods. The lower rod was connected to the base of the organ bath, while the upper rod was attached to the isometric force transducer which was coupled to Data Capsule Model 17400 for displaying isometric contractions. This was used in recording the force

we did not find any differences between the control, decaffeinated, kolanut extract and caffeine groups in the endothelium-independent relaxation (Fig. 3). Also, there was no significant difference in the maximum relaxation response of the aortic rings to the SNP in the presence of L-NAME in all the treated groups compared to control (Fig. 4).

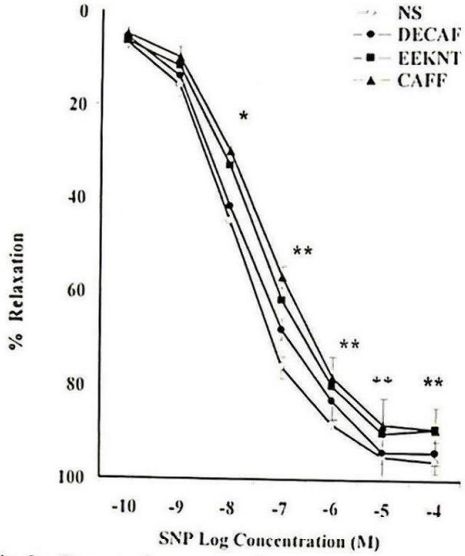

Fig. 3: Concentration-response for sodium nitroprusside -induced relaxation (SNP; 10^{-10} to 10^{-4}M) in rat aortic rings pre-contracted with noradrenaline (10^{-6} M) from Normal saline (NS), decaffeinated (DSCAF), ethanolic kolanut extract (EEKNT) and caffeine (CAFF) groups. Each point represents the mean ± SE (n=8). (*p<0.05; **p<0.01) compare with control group.

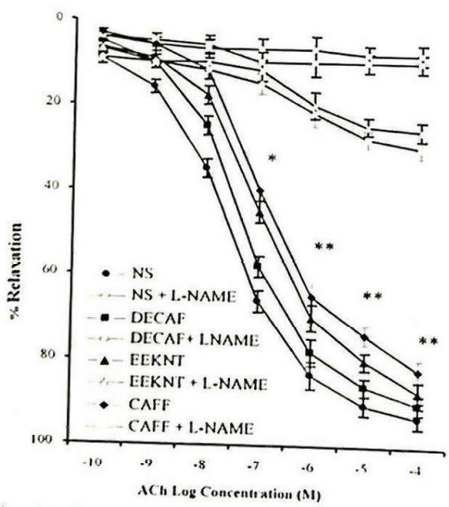

Fig. 4: Concentration-response for acetylcholine-induced relaxation (ACh; 10^{-10} to 10^{-4}M) in rat aortic rings pre-contracted with noradrenaline (10^{-6} M) in absence (filled symbols and continuous line) and presence of L-NAME (open symbol and dotted lines). Preparations were obtained from Normal saline (NS), decaffeinated (DSCAF), ethanolic kolanut extract (EEKNT) and caffeine (CAFF) groups. Each point represents the mean ± SE (n=8). (*p<0.05; **p<0.01) compare with control group.

Effect of Atropine, indomethacin, L-NAME or indomethacin plus L-NAME on vasorelaxation caused by ACh in aortic rings isolated from treated rats

The ACh-induced relaxation of the rat aortic rings was significantly reduced in the presence of L-NAME in caffeine and kolanut extract groups (Fig. 5). The presence of L-NAME was completely abolished the relaxation induced by ACh in the control and decaffeinated groups.

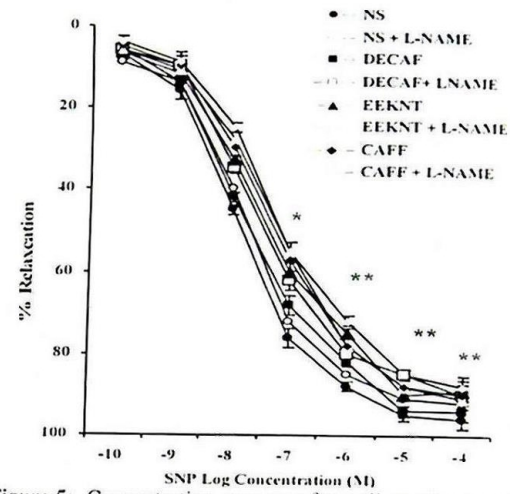

Figure 5: Concentration-response for sodium nitroprusside - induced relaxation (SNP; 10^{-10} to 10^{-4}M) in rat aortic rings pre-contracted with noradrenaline (10^{-6} mM) in presence of L-NAME from Normal saline (NS), decaffeinated (DSCAF), ethanolic kolanut extract (EEKNT) and caffeine (CAFF) groups. Each point represents the mean ± SE (n=8). (*p<0.05; **p<0.01) compare with control group.

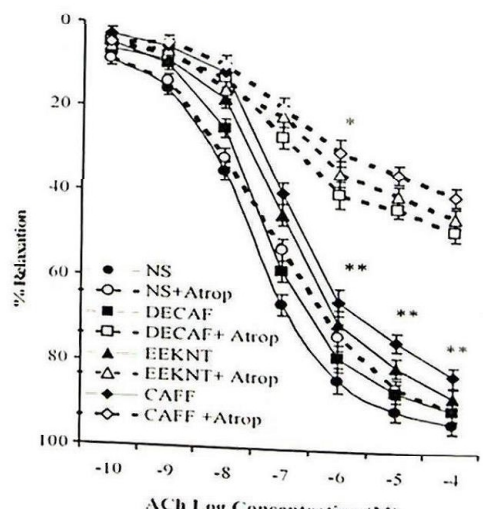

Fig. 6: Concentration-response for acetylcholine-induced relaxation (ACh; 10^{-10} to 10^{-4}M) in rat aortic rings pre-contracted with noradrenaline (10^{-6} M) in absence (filled symbols and continuous line) and presence of atropine (open symbol and dotted lines). Preparations were obtained from normal saline (NS), decaffeinated (DSCAF), ethanolic kolanut extract (EEKNT) and caffeine (CAFF) groups. Each point represents the mean ± SE (n=8). (*p<0.05; **p<0.01) compare with control group.

Table 2: Effect of Normal saline (NS), Decaffeinated kolanut extract (DECAF), ethanolic kolanut extract (EEKNT) and Caffeine (CAFF) on body weight (g) and mean arterial blood pressure (MABP) (mmHg) of rats. Values are presented as mean ± SE (n=10)

	Initial wt (g)	Final wt (g)	Weight gain (g)	% of wt gain	MABP (mmHg)
NS	223.8±6.5	249.4±3.9	25.8±4.8	11.8±2.5	116.4±2.01
DECAF	226.5±3.0	253±3.5	26.5±1.9	11.7±0.9	115.6±3.5
EEKNT	234.5±2.3	242.6±2.6	8.2±1.6	3.5±0.7*	137.3±1.4†
CAFF	227.1±2.1	238.8±1.07	11.9±1.6	5.2±0.7*	130.7±0.6†

†Significant increase ($p<0.05$) when compared with control and decaffeinated groups.
**Significant decrease ($p<0.05$) when compared with control and decaffeinated groups.*

The effect of the chronic consumption of kolanut extract, caffeine, and decaffeinated kolanut extract on contraction induced by NE (10^{-7}M) or KCl (80mM) in aortic rings

In this experiment the contractions were induced by a single concentration of noradrenaline (NE; 10^{-6} M) or KCl (80 mM) in aortic ring. Chronic consumption of decaffeinated kolanut, kolanut extract and caffeine for 6-weeks significantly ($p<0.05$) attenuated the noradrenaline-induced pre-contraction (Fig.1) or the KCl-induced contraction of rats aortic rings when compared with untreated control animals. The reduction in the contraction of decaffeinated kolanut extract group was significantly lower than caffeine groups. Also, a significant ($p<0.05$) difference was observed between the kolanut extract and caffeine-treated groups for both NE- and KCl- induced contractions when compared to control groups.

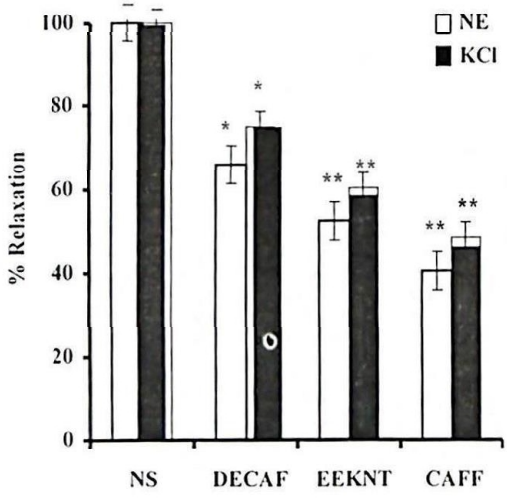

Fig.1: Contractions induced by the addition of a single concentration of noradrenaline (NE; 10^{-6} M) or KCl (80mM) in aortic rings obtained from normal control (NS), decaffeinated kolanut extract (DECAF), ethanolic kolanut extract (EEKNT), and caffeine (CAFF) groups. Each bar shows the mean ± SE of (n=8). (*$p<0.05$; **$p<0.01$) compared with control group

The Effect of the chronic consumption of kolanut extract, caffeine, and decaffeinated kolanut extract on the relaxation -induced by ACh or SNP in aortic rings

Acetylcholine (10^{-10} to 10^{-4} mM) caused an endothelium-dependent relaxation in a concentration –dependent manner in all the groups studied (Fig. 2). The maximum relaxation effects were 93%, 90%, 87% and 82% for normal saline (control), decaffeinated, kolanut extract and caffeine groups respectively. However, the sensitivity progressively decreased especially in the kolanut extract and caffeine groups.

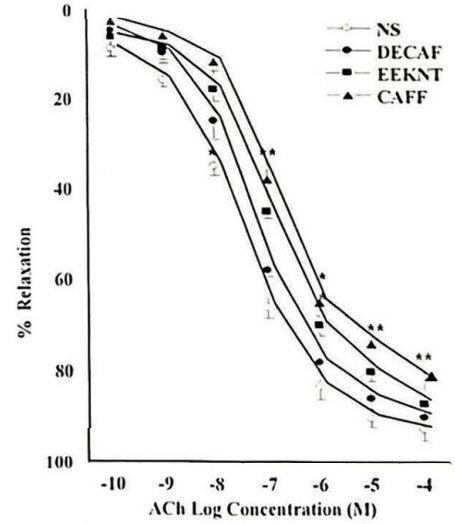

Fig. 2: Concentration-response for acetylcholine-induced relaxation (ACh; 10^{-10} to 10^{-4}M) in rat aortic rings pre-contracted with noradrenaline (10^{-6} M) from Normal saline (NS), decaffeinated (DSCAF), ethanolic kolanut extract (EEKNT) and caffeine (CAFF) groups. Each point represents the mean ± SE (n=8) (*$p<0.05$; **$p<0.01$) compared with control group.

Similarly, we also studied the possible changes in the endothelium-independent relaxation;

against hypertension [40]. These findings could partly explain the results observed in the present study.

The finding from this study indicates that chronic consumption of kolanut extract, decaffeinated and caffeine reduced the noradrenaline and high potassium-induced contraction of aortic rings. This observation is consistent with previous report that caffeine inhibits high K+ and NA – induced contraction in rabbit mesenteric artery [41]. It is well known that high K+/ NA –induced Ca^{2+} influx through voltage-dependent Ca^{2+} channel, via a receptor-mediated second messenger system [42] and activation of other Ca^{2+} channels receptors-operated Ca^{2+} channels by agonist has also been proposed [43]. In the present study chronic consumption of Kolanut extract and caffeine-induced relaxation responses in contraction produced by either of the agonist suggest that long-term consumption of kolanut , and caffeine blocks Ca^{2+} influx through interference with both voltage and receptors- operator channels. This observation is consistent with earlier reports that caffeine inhibits Ca^{2+} currents through voltage-dependent Ca^{2+} channels in rats cultured myometrium [44] and that these relaxation was also accompanied with decrease in Ca^{2+} [45]

Sato *et al,* [46] also reported that the inhibitory effects of caffeine was not only due to a decrease in Ca^{2+} but due to its direct effect on the contractile proteins. It is well known that caffeine act as a phosphodiesterase inhibitor and it increases the adenosine 3, 5 cyclic monophosphate content [38]. Caffeine increases cyclic adenosine monophosphate (cAMP) concentration in vascular tissues [47], this activate protein kinases, which phosphorylate myosin light chain kinase and more Ca^{2+} is added for activation, thus cAMP may decrease the Ca^{2+} sensitivity of the contractile element [48]. Decaffeinated kolanut extract analysis shows that it contain flavonoid and phenolic compound which have been shown to influence the vascular smooth muscle The reduction in the contraction induced by NA/ higher K+ observed in this study is in agreement with previous study who suggests that the relaxant action of the flavonoids includes inhibition of Ca^{+2} influx and release of Ca^{+2} from intracellular stores [49,50].

Another finding from this study is that 6weeks of treatment with kolanut extract, decaffeinated and caffeine did not significantly change the ACh-induced aortic relaxation. Similarly, the maximum relaxation induced with sodium nitroprusside was not different in kolanut extract, caffeine and decaffeinated treated groups. This finding suggests that NO is the most important endothelium-derived mediator involved in Ach-induced relaxation of the aortic rings. Meanwhile, the inhibitory effects of atropine on the ACh-induced relaxation suggest the involvement of cholinergic mechanism. This result is in agreement with our earlier report [51]. Also, since L- NAME, a nitric oxide synthase inhibitor abolished the kolanut extract-induced endothelium-dependent vasodilation, it confirms that kolanut extract stimulates endogenous nitric oxide production by agonist stimulation. It is well known that NO's play a pivotal role in the ACh –induced relaxation of the aortic ring [52,53]. The question remains whether ACh-induced relaxation of the aortic ring by the decaffeinated, kolanut extract and caffeine involves other endothelium derived mediators.

Our results show that ACh – induced relaxation of the aortic rings was attenuated in the presence of indomethacin and the ACh concentration-response curve was shifted significantly to the right. This indicates that prostanoids are involved in the vascular relaxation induced by ACh. This is in agreement with other reports which suggested the participation of endothelium-derived vasodilatation prostanoids in ACh-induced relaxation (54, 55) and are at variance with reports that indicate the involvement of prostanoid is negligible [56, 57].

Another report has shown that caffeine increases concentration of cAMP [47] and cAMP accumulation was also shown to induced NOS expression in the rat mesangial cells, with consequent enhancement of NO production and NO activated COX enzymes [58]. Therefore, it appears like a cross-talk between COX and NOS pathways may modify the effects of the ACh relaxation of the aortic rings.

We also verified the effect of chronic kolanut extract, decaffeinated and caffeine treatment on preparations treated with either indomethacin or L-NAME. Our methodological approach permits us to individually investigate the participation of either COX or NOS partway in ACh –induced aortic ring relaxation. The present results indicate that both COX and NOS pathways are unchanged in kolanut extract decaffeinated and caffeine treated animals. This suggests that caffeine, kolanut or decaffeinated treatment did not change ACh –induced aortic ring relaxation resistance to incubation with indomethacin plus L-NAME.

Recent studies have suggested that at a lower concentration caffeine induced actin

Treatment with atropine attenuated the ACh-induced relaxation of the rat aortic rings as well as shifting the ACh-concentration-response curve significantly to the right (Fig. 6). The ACh –induced relaxation of rat aortic rings was also significantly ($p<0.05$) reduced in the presence of indomethacin (Fig. 7). However, the ACh –induced relaxation was completely abolished when the preparation was incubated with indomethacin plus L-NAME (Fig. 8).

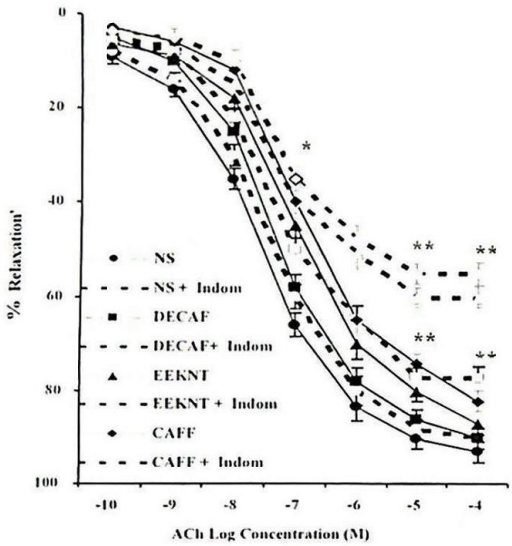

Fig. 7: Concentration-response for acetylcholine-induced relaxation (ACh; 10^{-10} to 10^{-4} M) in rat aortic rings pre-contracted with noradrenaline (10^{-6} M) in absence (filled symbols and continuous line) and presence of indomethacin (open symbol and dotted lines). Preparations were obtained from normal saline (NS), decaffeinated (DSCAF), ethanolic kolanut extract (EEKNT) and caffeine (CAFF) groups. Each point represents the mean ± SE (n=8). (*$p<0.05$; **$p<0.01$) compare with control group.

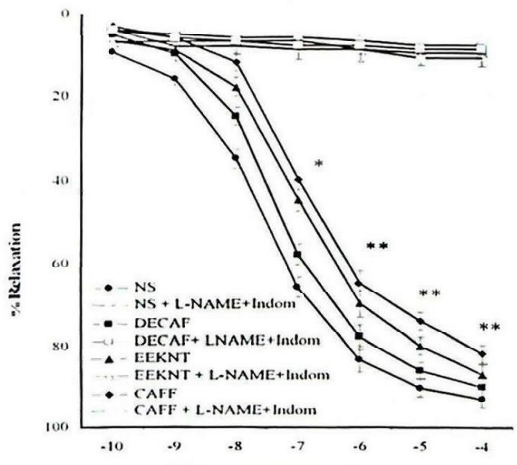

Fig. 8: Concentration-response for acetylcholine-induced relaxation (ACh; 10^{-10} to 10^{-4} M) in rat aortic rings pre-contracted with noradrenaline (10^{-6} M) in absence (filled symbols and continuous line) and presence of L-NAME plus indomethacin (open symbol and dotted lines). Preparations were obtained from normal saline (NS), decaffeinated (DSCAF), ethanolic kolanut extract (EEKNT) and caffeine (CAFF) groups. Each point represents the mean ± SE (n=8). (*$p<0.05$; **$p<0.01$) compare with control group

Discussion

The results of GC-MS indicate that the crude ethanolic extract of kolanut contains 51.1% of caffeine with about 97% pure. The caffeine contents of kolanut in this study differ from the previous reports by other workers. Somorin (28) found that *cola nitida* contains 0.16-gram caffeine per 100g powder of kolanut. Ogutuga [29] also reported that kolanut contains between 1–1.5% caffeine by weight of a dried powdered sample of kolanut [29]. Caffeine content in kolanut and coffee varies widely depending on the type of kolanut and the method of preparation used. Certain types of kolanut may contain somewhat more caffeine than others [29]. Besides, processing techniques may be accounted for the observed variation of caffeine content in kolanut [30].

Results from this study showed that chronic consumption of kolanut and caffeine resulted in a reduction in the body weight of rats. The greater increase in the final mean body weight of rats in the decaffeinated group when compared with the caffeine groups showed the reducing effects of caffeine on body weight. The observed reduction in the body weight by caffeine may be attributed to increased thermogenesis [31], lipolysis [32] and fat oxidation induced by caffeine [33].

In the present study, mean arterial blood pressure was elevated in crude kolanut extract, suggesting vasoconstriction effects of kolanut extract. Our results support those of previous studies showing that the chronic consumption of crude kolanut extract increased blood pressure [19, 20]. However, mean arterial blood pressure of caffeine groups were not different from the control group. It is known that acute administration of caffeine elevate blood pressure, but studies have shown that tolerance to caffeine develops rapidly [34] as heavy caffeine drinkers are less likely to develop an increase in blood pressure [35]. One of the mechanism by which acute caffeine administration increase blood pressure was through increasing circulating catecholamine [36] adenosine receptor antagonist [37] and activation of the rennin - angiotensin system [38]. Debrah *et al,* [39] reported that the increase in adrenaline levels induced by a single dose of caffeine will be reduced with the sustained consumption of more caffeine. Even the recent epidemiological studies suggest a protective role of high coffee intake

21. Chukwu LO, Odiete WO, and Briggs LS. Basal metabolic regulatory responses and rhythmic activity of mammalian heart to aqueous kola nut extracts. African Journal of Biotechnology. 2006; 5: 484-486.
22. Rossenthaler L. The chemical investigation of plants. Translated into English by Sudhamoy Ghosh from the Third_German edition. 1930; Bell and Sons. Ltd Lond.pp 5-6
23. Murray DS and Hansen PJ. Extraction of Caffeine from tea. J.Chem. Educ. 1995; 72: 851.
24. Hampp A. Extraction of Caffeine from plants J. Chem. Educ., 1996; 73: 1172.
25. Nawrot P, Jordan S and Eastwood J. Effects of caffeine on human health. Food Addit Contam 2003; 20: 1 – 30.
26. Donovan JL and DeVane CL. A primer on caffeine pharmacology and its drug interactions in clinical psychopharmacology. Psycho pharmacol Bull 2001; 35: 30 – 48.
27. Salahdeen HM and Alada ARA. Effects of caffeine and ethanolic extract of kolanut on glucose uptake in the canine hindlimb at rest and during contraction. Nigerian Journal of Physiological Sciences. 2009; 24: 33-45.
28. Somorin O. Spectrometric determination of caffeine in Nigerian Kolanuts. Journal of Food Sciences, 1973; 381: 911-913.
29. Ogutuga DBA. Chemical composition and potential commercial uses of kolanuts, Cola nitida vent (Schott and Endlisher) Ghana Journal of Agricultural Sciences 1975; 8: 121-125.
30. Arogba SS. Studies on kolanut and cashew kernels: moisture adsorption isotherm, proximate composition and functional properties. Food Chemistry. 1999; 67: 223-228.
31. Hicks MB, Hsieh YH and Bell LN. Tea preparation and its influence on methyl-xanthine concentration. Food Research International, 1996; 29: 325-330.
32. Zheng G, Sayama K and Okubo T. Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine in mice. *In Vivo*. 2004; 18: 55-62.
33. Lopez-Garcia E, van Dam RM and Rajpathak S. Changes in caffeine intake and long-term weight change in men and women. Am J Clin Nutr. 2006; 83: 674–680.
34. Greenberg JA, Axen KV and Schnoll R. Coffee, tea and diabetes: the role of weight loss and caffeine. Int J Obes (Lond). 2005; 29: 1121–1129.
35. Robertson D, Wade D and Workman R. Tolerance to the humoral and hemodynamic effects of caffeine in man. J Clin Invest 1981; 67: 1111–1117.
36. Myers MG. Effects of caffeine on blood pressure. Arch Intern Med 1988; 148: 1189-1193.
37. Benowitz NL. Clinical pharmacology of caffeine. Annu Rev Med. 1990; 41: 277–288.
38. Fredholm BB, Battig K and Holmen J. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. Pharmacol Rev 1999; 51: 83–133.
39. Debrah K, Haigh R and Sherwin R. Effect of acute and chronic caffeine use on the cerebrovascular, cardiovascular and hormonal responses to orthostasis in healthy volunteers. Clin Sci (Lond) 1995; 89: 475- 480.
40. Geleijnse JM. Habitual coffee consumption and blood pressure: an epidemiological perspective. Vasc Health Risk Manag 2008; 4: 963-970.
41. Ito Y., Suzuki H. and Kuriyama H. Effects of caffeine and procaine on the membrane and mechanical properties of smooth muscle cells of the rabbit main pulmonary artery. Journal of Pharmacology. 1977;27: 467-481.
42. Nelson T., Standem B., Brayden E. and Worley F. Noradrenaline contracts arteries by activating voltage-dependent calcium channels. Nature 1988; 336: 382-385.
43. Bolton T. B. Mechanisms of action of transmitters and other substances on smooth muscle. Physiological Reviews 1979 ; 59 : 607-718.
44. Martin C, Dacquet C., Mironneau C and Mironneau, J. Caffeine induced inhibition of calcium channel current in cultured smooth muscle cells from pregnant rat myometrium. British Journal of Pharmacology 1989; 98: 493-498.
45. Watanabe C., Yamamoto H., Hirano,*et al*. Mechanisms of caffeine- induced contraction and relaxation of rat aortic smooth muscle. J. *Physiol*. 1992; 456: 193–213.
46. Sato K., Ozaki H. and Karaki H. Multiple effects of caffeine on contraction and cytosolic free Ca2+ levels in vascular smooth muscle of rat aorta. Archives of Pharmacology 1988; 338: 443-448.
47. Bray K. M., Longmore J. and Weston A. H. Analysis of caffeine-induced responses in rabbit isolated aorta. Journal of Physiology 1989; 410: 77P.
48. Nishimura J. and Van Breemen C. Direct regulation of smooth muscle contractile elements. Biochemical and Biophysical Research Communications 1989;163: 929-935..

depolymerisation disrupts cross-bridge formation in the intact and permeabilized smooth muscle tissues, leading to a relaxation, without affecting myosin motor function [59,60]. It is noteworthy that lower concentration of caffeine was used in this study (6 mg/kg/body weight) which is equivalent to three cups of coffee per day in human [24], and one cup is equivalent to 227 g of regular coffee, which contains 137 mg of caffeine [25].

In conclusion, treatment with decaffeinated kolanut extract and caffeine does not change ACh – induced aortic ring relaxation. The vasodilatory effect of decaffeinated kolanut extract may be due in part to the presence of flavonoids, including quercetin in this extract. Similarly, the effects of kolanut extract on vascular smooth muscle are essentially similar to that of caffeine in this study, it will therefore be in place to conclude that the effects of kolanut extract is being carried out by the presence of caffeine in it. Further studies whereby different concentrations of kolanut extract, caffeine, decaffeinated kolanut and other active substances isolated from kolanut extract will throw more light on the mechanisms of action of kolanut on the cardiovascular functions in rat.

References

1. World Health Organization. The World Health Report 2002: Reducing risks, promoting healthy life. 2002; Geneva, Switzerland.
2. Kaerney PM, Whelton M, Reynolds SK, *et al.* Global burden of hypertension: an analysis of world wide data. Lancet.2005; 365: 217-223.
3. Rakic V, Burke V and Berlin LJ. Effects of caffeine on ambulatory blood pressure in older men and women: a randomized control trial. Hypertension. 1999; 33: 869-873
4. van Dam RM, Willett WC and Manson JE. Coffee, caffeine, and risk of type 2 diabetes: a prospective cohort study in younger and middle-aged U.S. women. Diabetes Care. 2006; 29: 398 – 403.
5. James JE. Is habitual caffeine used: A preventable cardiovascular risk factor? Lancet. 1997; 349: 279-281.
6. Pizziol, A, Tikhonoff, V and Paleari CD. Effects of caffeine on glucose tolerance: a placebo-controlled study. Eur J Clin Nutr. 1998; 52: 846-849.
7. Keijzers GB, De Galan, BE, Tack, CJ, and Smits P. Caffeine can decrease insulin sensitivity in humans. Diabetes Care. 2002; 25: 364-369.
8. van Dam RM, and Feskens EJ. Coffee consumption and risk of type 2 diabetes mellitus. Lancet. 2002; 360: 1477-1478.
9. Ross GW, Abbott RD and Petrovitch H. Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA*. 2000; 283:2674-2679.
10. Zeegers MP, Tan FE, Goldbohm RA and van den Brandt PA. Are coffee and tea consumption associated with urinary tract cancer risk? a systematic review and meta-analysis. Int J Epidemiol. 2001; 30:353-362.
11. Ferrini RL and Barrett-Connor E. Caffeine intake and endogenous sex steroid levels in postmenopausal women: the Rancho Bernardo Study. Am J Epidemiol. 1996; 144: 642-644.
12. Nagata C, Kabuto M. and Shimizu H. Association of coffee, green tea, and caffeine intakes with serum concentrations of estradiol and sex hormone-binding globulin in premenopausal Japanese women. Nutr Cancer. 1998; 30: 21-24.
13. Goodman-Gruen D, and Kritz-Silverstein D. Usual dietary isoflavone intake is associated with cardiovascular disease risk factors in postmenopausal women. J Nutr. 2001; 131: 1202-1206.
14. Leitzmann MF, Willett WC, and Rimm EB. A prospective study of coffee consumption and the risk of symptomatic gallstone disease in men. JAMA. 1999; 281: 2106-2112.
15. Curhan GC, Willett, WC, Speizer FE and Stampfer MJ. Beverage use and risk for kidney stones in women. Ann Intern Med. 1998;128: 534-540.
16. Kleemola P, Jousilahti P, Pietinen P, Vartiainen, E, and Tuomilehto J. Coffee consumption and the risk of coronary heart disease and death. *Arch Intern Med.* 2000; 160: 3393-3400.
17. Robertson D, Hollister AS and Kincaid D. Caffeine and hypertension. Am J Med. 1984; 77: 54-60.
18. Beilin LJ, Puddey IB and Burke V. Lifestyle and hypertension. Am J Hypertens. 1999; 12: 934 - 945.
19. Osim EE, and Udia PM. The effect of consuming kola nut (*Cola nitida*) diet on mean arterial pressure in rats. Int. J. Pharmacog. 1999; 31: 193-197.
20. Ighinovia ENS, Ugwu AC, Nwaopara AO, Otamere HO and Adisa WA. The Effects of *Cola acuminata* on arterial blood pressure. Pakistan Journal of Nutrition 2009; 8: 148-150.

49. Ajay M, Gilani AH and Mustafa MR. Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. Life Sci. 2003; 74: 603–612.
50. Rice-Evans A. and Packer L. Flavonoids in Health and Disease, Marcel Dekke Inc.,1998; New York. Pp 55.
51. Salahdeen H M Yemitan O K, Alada A R A. Effect of aqueous leaf extract of *Tridax procumbens* on blood pressure and heart rate in rats. Afri. J. Biomed. Res. 2004; 7: 27-29.
52. Sekiguchi F, Miyake Y, Hirakawa A, *et al.* Hypertension and impairment of endothelium-dependent relaxation of arteries from spontaneously hypertensive and L-NAME-treated Wistar rats. J. Smooth Muscle Res. 2001;37: 67–79.
53. Woodman O L, and Boujaoude M. Chronic treatment of male rats with daidzein and 17betaoestradiol induces the contribution of EDHF to endothelium-dependent relaxation. Br. J. Pharmacol. 2004;141: 322–328.
54. Yang B C, Lawson DN and Mehta J L. Role of eicosanoids in rat aortic ring response to agonists and acetylcholine with special reference to the biphasic effects of prostacyclin. Eicosanoids 1992; 5: 135–139.
55. Callera G E, Varanda W A and Bendhack L.M. Impaired relaxation to acetylcholine in 2K-1C hypertensive rat aortas involves changes in membrane hyperpolarization instead of an abnormal contribution of endothelial factors. Gen. Pharmacol. 2000; 34: 379–389.
56. Bobadilla RA, Henkel CC, Henkel EC, *et al.* Possible involvement of endothelium-derived hyperpolarizing factor in vascular responses of abdominal aorta from pregnant rats. Hypertension 1997; 30: 596–602.
57. Tomioka H, Hattori Y, Fukao M, *et. al.* Relaxation in different-sized rat blood vessels mediated by endothelium-derived hyperpolarizing factor: importance of processes mediating precontractions. J. Vasc. Res.1999; 36: 311–320.
58. Tetsuka T, Daphna-Iken D, Srivastava S K, *et al.* Crosstalk between cyclooxygenase and nitric oxide pathways: prostaglandin E2 negatively modulates induction of nitric oxide synthase by interleukin 1. Proc. Natl. Acad. Sci. USA. 1994; 91: 12168–12172.
59. Dyrda P, Tazzeo T, DoHarris L, *et al.* Acute response of airway muscle to extreme temperature includes disruption of actin-myosin interaction. Am. J. Respir Cell Mol biol. 2011; 44: 213-221.
60. Tazzeo T, Bates G, Roman HN, *et al.* Caffeine relaxes smooth muscle through actin depolymerisation. Am J Physiol. 2012; 303: L334 - L342.

a saddle on one side of the arch with other components on the other side [1-3]. In addition to its basic role, major connectors may contribute to the support and bracing of a denture. It may also help to retain the denture by providing indirect retention [1, 4], or occasionally acts as a splint and provides a stabilizing effect on periodontally weakened anterior teeth [5].

Several studies have shown that removable partial dentures can predispose to or aggravate existing periodontal diseases [6-8]. This is because it enhances plaque accumulation and favours the growth of more pathogenic plaque bacteria. Chamrawy [6], found that consistently higher plaque scores were obtained from all teeth surfaces during denture wearing. Arigbede *et al* [7], in a study on Nigerians while assessing the impact of major connectors on gingival health, reported a significant increase in the mean plaque index of the abutment teeth but did not identify any significant increase in the control teeth. He concluded that metallic partial dentures appear to cause an increased plaque accumulation and gingival inflammation around abutment teeth than the control teeth.

The effect of various components of the removable partial denture on periodontal health has been investigated by various authors [8, 9]. The clasp arm was reported to cause more plaque and calculus accumulation on the abutment teeth when compared with non abutment teeth [8, 9]. McHenry *et al* [10], evaluated the changes in the health of the gingival tissue using the lingual plate and cingulum bar connectors, and reported a greater increase in gingival inflammation when a lingual plate was used when compared with a bar connector. Also, Zlateric *et al* [8], assessed the oral health of Removable Partial Denture (RPD) wearers and discovered that the highest plaque index, calculus index, gingival recession, probing depth were registered in lingual plate RPD's.

The sequelae of the coverage of the marginal gingival results in an increased plaque accumulation and gingivitis, and the severity depends on the medical condition and oral hygiene of the patient, the host response to plaque, the alignment of the natural teeth, and the duration of the coverage [10].

Removable partial dentures are the most common option of replacing missing teeth amongst partially edentulous Nigerian patients [11]. However, these patients demonstrate a poor clinic attendance especially for review and this necessitated the need to incorporate components that reduces plaque retention. The lingual bar and plate major connectors form an important component of the mandibular removable partial dentures, and there appear to be limited literature in our environment on their influence on gingival health. This calls for more studies to assess the effect of this major component of the metallic RPD on periodontal health. This study was therefore designed to assess the influence of lingual bar and plate major connectors on gingival health among patients in University College Hospital (UCH), Ibadan, Nigeria.

Materials and methods

The study was conducted at the prosthetic outpatient clinic of the Dental Centre, University College Hospital (UCH), Ibadan, Nigeria.

The sample size was calculated using the formula

$$N = \frac{(Z\alpha + Z\beta)^2 (S/2)^2}{(Q1 - Q2)^2}$$

Where N= minimum sample size, $Z\alpha$ = standard normal deviate corresponding to the probability of type I error (α) at 5% = 1.96

$Z\beta$ = standard normal deviate corresponding to the probability of type II error (β) of 20%. Power at 80% = 0.84

S = Standard deviation, Q1 = Mean score 1, Q 2 = mean score 2

Using mean gingival index score of 0.8 (± 0.9) and 1.4 (± 0.7) for lingual bar and plate respectively[12]

$$N = \frac{[1.96 + 0.84]^2 [(0.9 + 0.7)/2]^2}{[1.4 - 0.8]^2}$$

$$= 13.938$$

$$= 14 \text{ Patients}$$

Fifteen out of eighteen patients that were selected participated fully in the study. Three patients did not turn up for further management after the try-in of metal frame work. Selection was made according to the following criteria: volunteers with no systemic disease, non smokers, healthy periodontal tissue, Kennedy class III modification 1 or 2 partially edentulous mandibular arch, absence of dental caries, minimum depth of lingual sulcus of 7mm, and no history of wearing of removable partial dentures prior to this study. Exclusion criteria included poor oral hygiene, high frenal attachment, presence of lingual tori, malocclusion such as rotation or imbrications of the natural teeth and an inadequate depth of the lingual sulcus of less than 7mm. Kennedy class III patients were incorporated into the study population to ensure that the design of the dentures were essentially similar except for the bar and plate connector system for each patient.