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The Administration of Goroho Banana (*Musa Acuminata Sp.*) Peel Extract Reduces Weight, Increases Number of Leydig Cells and Testosterone Level in Male Wistar Rats (*Rattus norvegicus*) with Obesity

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ABSTRACT

Oxidative stress can cause obesity, reduce both the number of Leydig cells and serum testosterone levels, thus triggering aging process. Goroho banana peel (*Musa acuminata sp*.) contains antioxidants to neutralize free radicals to maintain organ functions in a physiological state and avoiding the risk of premature aging. This study aims to prove that administration of goroho banana peel extract reduces body weight, increases the number of Leydig cells and testosterone levels in male Wistar rats (*Rattus norvegicus*) exposed to obesity.

This was experimental research using post-test only control group design. The subjects were 36 male Wistar rats with obesity, aging 2-3 months, randomly divided into 2 groups namely the control group and treatment group. Control group was given placebo in the form of 1 ml distilled water while the treatment group was given goroho banana peel extract. The result was analyzed using independent t-test after 28 days.

According to Lee's index criteria, the total average weight in control group was 0,31±0,008, while the treatment group was 0,26±0,01, with p=0.001 (p<0.05). The mean number of Leydig cells in the control group was 15.31±2.06/LPB, while the treatment group was 29.13±1.45/LPB with p=0.001 (p<0.05). The mean testosterone level in the control group was 3.71±0.02 nmol/mL, while the treatment group was 7.92±0.30 nmol/mL (p=0.001 (p<0.05)). There were significant differences between the control group and the treatment group in body weight, Leydig cell count, and testosterone level.

The conclusion of this study was that the administration of goroho banana peel extract (*Musa ≥acuminata sp*.) reduces body weight and increases the number of Leydig cell and testosterone level in male Wistar rats (*Rattus norvegicus*) with obesity.

Keywords: Antioxidants, goroho banana peel extract, obesity, weight loss, Leydig cell, testosterone level

INTRODUCTION

Obesity is a global epidemic problem that must be addressed immediately (Body Mass Index (BMI) ≥25kg/m^2 (Kementerian Kesehatan RI, 2018). Obesity is caused by fat accumulation, due to disorders of lipogenesis, lipolysis, free radicals, genetics, unhealthy lifestyle, drugs and stress (Fui *et al*., 2014). Testosterone is an androgen hormone that can be found in men and women. In men, 95% of testosterone is produced by Leydig cells in the testes and 5% by the adrenal cortex (Pangkahila, 2017). Decreased testosterone (≤300 ng/dL) is called hypotestosterone, which can be caused by aging, obesity, decreased Leydig cells, oxidative stress, endocrine disorders, decreased bioavailability of testosterone. Hypotestosterone can interfere with the reproductive system (sexual dysfunction and spermatogenesis), systemic (obesity, cardiovascular disease, increased fracture risk), and decrease the patient's quality of life (Decroli, 2022; Pangkahila, 2017; Roychoudhury *et al*., 2021; Wardana, 2016).

According to the Cohen Hypothesis regarding the Hypogonadal Obesity Cycle, testosterone is converted to 17β estradiol (E2) by the aromatase enzyme in adipose tissue while testosterone production is suppressed. When adipose is high, aromatase conversion increases so that testosterone decreases. Decreasing testosterone increases adipose but also further decreases testosterone. (Decroli, 2022; Wardana, 2016). Antioxidant compounds work by inhibiting auto-oxidation of lipids or other molecules by inhibiting the initiation or spread of chain oxidative reactions, so that these compounds can prevent damage to cells (Radical *et al*., 2011).

According to study from Rusmini *et al*., administration of plant extracts that contain antioxidants, namely flavonoids, tannins, saponins, phenolics, alkaloids, menoterpenes, sesquiterpenes, quinones can reduce Lee's obesity index by 4.46% - 11.94% (Rusmini *et al*., 2021). The dose of ethanol extract of 6.3 g/kg BW of goroho banana peel used in this study was based on previous studies because it contains secondary metabolic compounds of flavonoids,

saponins, tannins as antioxidants which have been shown to be effective in significantly reducing blood glucose in male Wistar rats (Syamsuddin *et al*., 2013). A study from Permatasari showed that exposure to free radicals for 28 days significantly reduced Leydig cells and serum testosterone level (Permatasari, 2021).

According to Arias, bananas are most frequently searched for fruits in scientific publications, as antioxidant number 10 compared to other fruits (Arias, 2022). Sathya stated that the goroho banana species (*Musa acuminata sp*.) has the highest antioxidants (vitamin C, peroxidase, and catalase) compared to other species (Sathya, 2014). Phytochemical analysis of antioxidants from goroho banana peel shows that it has the highest DPPH (1,1–diphenyl-2 picrylhydrazyl) free radical scavenger than other parts such as fruit, seeds, flowers, leaves, midrib, heart, sap and roots (Alhabsyi *et al*., 2014).

Based on the antioxidant content in the goroho banana peel, it is very good to be processed as an extract in reducing obesity weight, increasing the number of Leydig cells, and increasing the hormone testosterone. Because it has been explained that obesity with a decrease in the number of Leydig cells and testosterone levels is still related to the role of oxidative stress. From the description above, the researchers tried to study and prove the effect of goroho banana peel extract (*Musa acuminata sp*.) in reducing body weight, increasing the number of Leydig cells and testosterone in obese male Wistar rats (*Rattus norvegicus*).

METHODS

Study Design and Experimental Animals

This research is an experimental study with post-test only control group design. The production of goroho banana peel extract was done at Food Technology Laboratory, Udayana University. Phytochemical and antioxidant screening took place at Faculty of Agricultural Technology, Biochemistry and Nutrition Laboratory, Udayana University. The experiment and testosterone level examination was carried out at the Integrated Biomedical Laboratory of Udayana University, while Leydig cell examination was carried out at Pathology Anatomy Laboratory of PTN Hospital, Udayana University, Jimbaran. The sample needed in this experiment was 32 male Wistar rats (n=16), 2-3 months old with obesity (based on Lee's index criteria >0.300). To anticipate drop out, 10% of total sample were added, with the total amount to 36 rats divided into 2 groups: control and treatment group (n=18). This research has been approved by the ethics commission of Udayana University, Bali, (B/240/UN14.2.9/PT.01.04/2022).

Goroho Banana Peel Extract Production

As much as 5 kg raw goroho banana peels were cleaned thoroughly, squeezed so that the sap came out, then the skin was cut into smallest pieces, and 2.5 kg of it was obtained. It was then macerated for 48 hours with 96% ethanol with a ratio of 1:9, then filtered with Whatman paper no 1. The filtrate was evaporated with a rotary evaporator and 820g thick extract was obtained. The goroho banana extract was concentrated so it was diluted with distilled water 1:1 and given 2 times a day.

Experimental Animal Treatment

Wistar rats used as experimental animals were adapted in individual cages for 7 days before treatment and given a diet high in fat and carbohydrate for 4 weeks to induce obesity based on Lee's index criteria. After 4 weeks, 36 obese rats were selected and randomly divided into two groups namely control and treatment group. In the control group (P0), the rats were given placebo of 1 mL of distilled water, twice per day. In treatment group (P1), the rats were given as much as 6.3g/kgBW/day of goroho banana peel extract for 28 days. After 28 days of treatment, all obese rats were weighed then euthanasia was performed using 10% ketamine at a dose of 50mg/kg and 2% xylazine at a dose of 20mg/kg. 1 ml of blood was drawn to check the testosterone level, and surgery was performed to take testicular samples for histological examination of Leydig cell.

Testosterone Hormone Examination

Testosterone level were checked with ELISA method with following steps: 50 µl of standard, sample, and quality control were put into the wells. 100 µl of Testosterone-HRP Conjugate was added into each well, except for quality control wells and standards, shook for 1-2 minutes and incubated for 60 minutes in room temperature (37°C). The liquid was discarded and washed with 250µL wash buffer solution 5 times, turn over the plate and the liquid residue was dried with a paper towel. Add 100 µL of TMB substrate solution to each well, and incubate the plate for 20 minutes at room temperature. Add 50 µL stop solution and the solution was homogenized for 30 minutes, the color of the solution changed from blue to yellow. Microwells were read with a spectrophotometer at a wavelength of 450nm.

Leydig Cell Examination

The process of making histological preparations were as followed: (1) Trimming, the specimens were fixed in buffered formalin, washed with running water and cut with thickness of 2-4mm then inserted into the embedding cassette and washed. (2) Dehydration, using 70% alcohol for 10 minutes, 80%, 90%, 96% alcohol for 60 minutes each, and absolute alcohol for 30 minutes. (3) Embedding, the tissue in the cassette was transferred to the base mold, filled with liquid paraffin then placed on a 3x3 cm wooden block. (4) Cutting, the block that had been cooled before was cut with 6 microns thickness. The best tissue sheet was chosen, float it in the water and remove the wrinkles. Transfer the tissue sheet to a water bath then place in the center of the slide and place in an incubator for 24 hours. (5) Staining with Haematoxilin-Eosin. (6) Mounting, place the slide on a tissue paper, dripped with Canada balsam and covered with cover glass. (7) Observed with binocular microscope.

Statistical Analysis

Statistical analysis was performed with SPSS. Normality test was assessed using Shapiro-Wilk test and homogeneity test was assessed with Levene's test. Comparability test was assessed using t-independent if the data was normally distributed and Mann Whitney if the data was non-normally distributed.

RESULTS

Normality test on Lee index criteria, Leydig cell number and testosterone level was done using Shapiro-wilk test, presented on Table 1. The data was normally distributed.

Table 1. Normality test

Variable	Subject Group	n	p	Desc
Lee Index	Control	18	0,23	Normal
	Treatment	18	0,66	Normal
Leydig cell	Control	18	0,12	Normal
(/FOV)	Treatment	18	0,34	Normal
Testosterone level (nmol/mL)	Control	18	0.44	Normal
	Treatment	18	0.71	Normal

n = sample; p = significance

Levene's homogeneity test is presented in Table 2. Based on Table 2, it was concluded that data variety of Leydig cell was homogeny ($p>0.05$), while the data variety of testosterone level and Lee index was not homogeny ($p<0.05$).

Table 2. Homogeneity test with Levene's Test

Variable	n	P	Homogeneity
Lee index	36	0.018	Not Homogeny
Leydig cell (/FOV)	36	0.290	Homogeny
Testosterone level (nmol/mL)	36	0.018	Not Homogeny

n = samples; p= significance

Comparability test to compare the average of Lee index, Leydig cell and testosterone level was done using t-independent test. The result of comparability test is presented in Table 3.

Table 3. Comparability Test

Variables	Group		p
	Control Mean±SB	Treatment Mean±SB	
Lee index	0.31±0.008	0.26±0.01	0.001
Leydig cell (/FOV)	15.31±2.06	29.13±1.45	0.001
Testosterone level (nmol/mL)	3.71±0.02	7.92±0.30	0.001

p = significance

The independent t-test showed that average body weight (Lee's index) in the treatment group was lower than control group and is shown in Figure

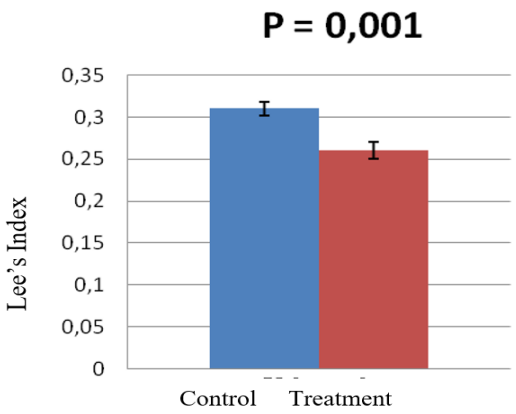

Figure 1. Comparison on Lee's Index between Groups

The independent t-test showed that mean Leydig cell in the treatment group was higher than control group and is shown in Figure 2.

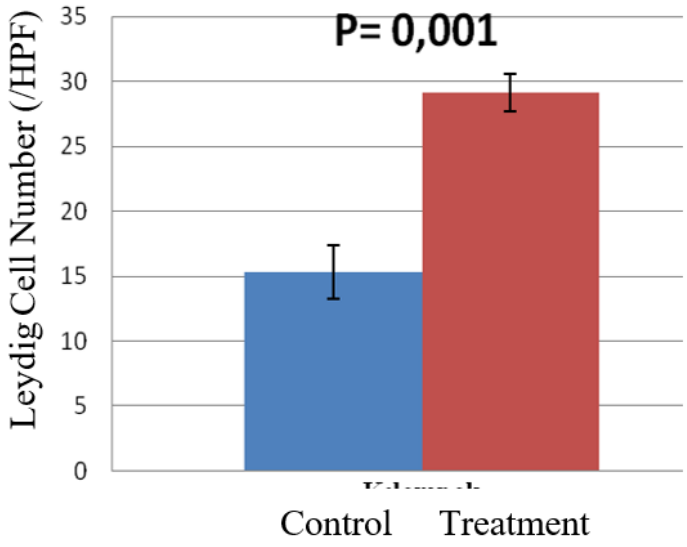

Figure 2. Comparison on Leydig Cell's Number between Groups

Figure 3 showed the histological appearance of round or polygonal Leydig cells with a central nucleus and eosinophilic cytoplasm, and the number of Leydig cells in the control group (K1) is less than the number of Leydig cells in the treatment group (P1).

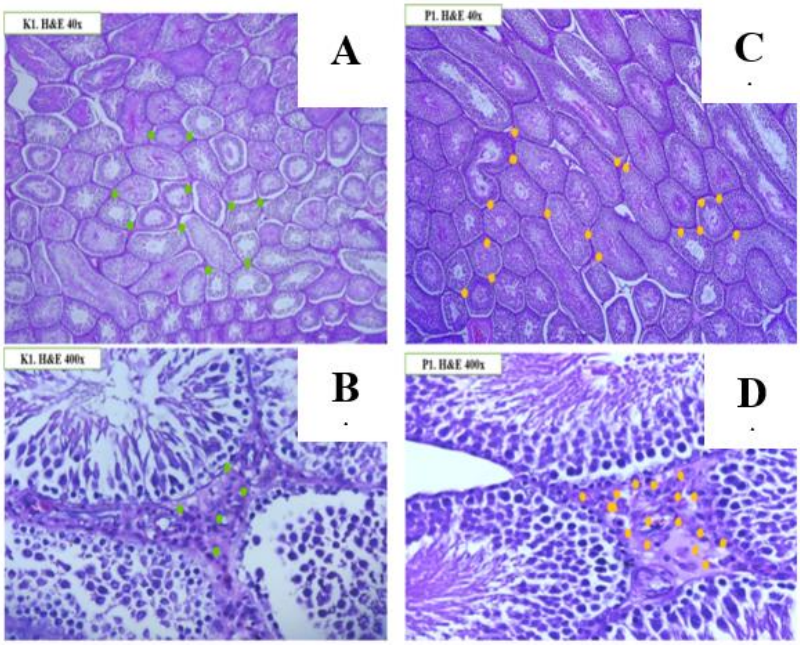

Figure 3. Histological preparation of Leydig cells (Hematoxylin-Eosin staining, distilled water was shown with green dots and goroho peel extract was shown with yellow dots). A: K1, 40x magnification. B: K1, 400x magnification. C: P1, 40x magnification. D: P1, 400x magnification

The independent test showed that mean testosterone level in the treatment group was lower than control group and is shown in Figure 3.

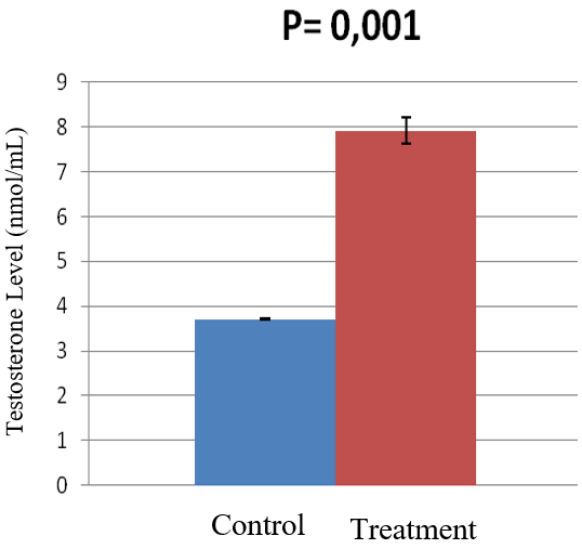

Figure 4. Comparison on Mean Testosteron Level between Groups

DISCUSSION

The Effect of Obesity on Leydig Cell Count and Testosterone Level

The results of the data analysis test showed that the mean number of Leydig cells in male Wistar rats (*Rattus norvegicus*) in the control group was 15.31±2.06/FOV. According to Novita, the average number of Leydig cells in male Wistar rats, aged 2-3 months, is 24.75 / LPB (Novita, 2021). The decrease in Leydig cells can be caused by obesity which triggers oxidative stress due to accumulation of ROS resulting in apoptosis of Leydig cells (Chen *et al*., 2016; Tang *et al*., 2014).

The average of testosterone levels in male Wistar rats in the control group in this study was 3.71 ± 0.02 nmol/mL. According to Khatimah, the average of normal testosterone level in male Wistar rats (Rattus norvegicus) at the age of 2-3 months is 4-6 nmol/mL (Khatimah, 2015). In obesity, testosterone is converted to 17β oestradiol (E2) by the aromatase enzyme in adipose tissue while its production is suppressed. When adipose is high, aromatase conversion increases so that testosterone decreases. Based on the results of this study, the control group's testosterone levels were lower than the normal average, which indicates that obesity can reduce the control group's serum testosterone levels.

The occurrence of oxidative stress in the body can damage toxic defenses against biological molecules/cells, DNA production, cell wall lipid layers, blood vessels, prostaglandin production, and other proteins such as enzymes contained in the body and change the antioxidant defense mechanism in the body thereby reducing activity of antioxidant enzymes such as SOD, CAT, Glutathione (Parwata, 2016). As a result of oxidative stress that occurs in obesity, it causes apoptosis of testicular Leydig cells, resulting in a decrease in the number of Leydig cells so that testosterone production by testicular Leydig cells decreases (Chen *et al*., 2016).

Administration of Goroho Banana Peel Extract (*Musa acuminata sp.*) Reduces Weight (Lee's Index), Increases Leydig Cell Count and Testosterone Level

The results of data analysis on body weight (Lee's index), showed that the mean in the control group was 0.31±0.008 g/cm, which was higher than the treatment group (0.26±0.01 g/cm). The results of the data analysis test for Leydig cell number showed that the mean in the control group was 15.31±2.06/FOV, which was lower than the treatment group (29.13±1.45/FOV). Meanwhile, the average testosterone level in the control group was 3.71±0.02/FOV, which was lower than the treatment group (7.92±0.30/FOV). Based on the results of this study can be concluded that administration of goroho banana peel extract can overcome oxidative stress thereby reducing body weight (Lee index), increasing the number of Leydig cells, and increasing testosterone levels in male Wistar rats with obesity. The results of this study are in line with research conducted by Rusmini *et al*., that administration of several extracts from plants containing antioxidant compounds such as flavonoids, tannins, saponins, phenolics, alkaloids, menoterpenes, sesquiterpenes, quinones can reduce Lee's obesity index by 4.46% -11.94% (Rusmini *et al*., 2021).

Benefits of Goroho Banana Peel Extract (*Musa acuminata sp.*) Against Anti-Aging Medicine

The antioxidants possessed by the peel of goroho banana are exogenous antioxidants derived from plants. From the antioxidant phytochemical analysis, goroho banana peel has the highest value of DPPH (1,1–diphenyl-2-picrylhydrazyl) as a free radical scavenger compared to other parts such as fruit, seeds, flowers, leaves, heart, sap and roots (Alhabsyi *et al*., 2014).

The results of this study showed that there were significant differences in weight loss (Lee index) and the increase in the number of Leydig cells and testosterone levels which was very likely due to the effectiveness of goroho banana peel as an antioxidant through the mechanism of inhibiting oxidative stress due to obesity exposure.

CONCLUSION

Goroho banana peel extract (*Musa acuminata sp*.) reduces body weight and increases Leydig cell number and testosterone level in male Wistar rats (*Rattus norvegicus*) with obesity.

Conflict Of Interest

All researchers declare that there is no conflict of interest related to this article

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All authors contribute equally in compiling this research article

REFERENCES

Alhabsyi DF, Suryanto E, Wewengkang DS. Aktivitas Antioksidan dan Tabir Surya pada Ekstrak Kulit Buah Pisang Goroho (*Musa acuminate L.*). Jurnal Ilmiah Farmasi 2014;3(2).

Arias A, Feijoo G, Moreira MT. Exploring the Potential of Antioxidants from Fruits and Vegetables and Strategies for Their Recovery. Innovative Food Science and Emerging Technologies 2022;77.

Chen J, Wang Y, Hui C, Xi Y, Liu X, Qi F *et al*. Mechanisms of Heshouwuyin in Regulating Apoptosis of Testicular Cells in Aging Rats Through Mitochondrial Pathway. Bmc Complementary and Alternative Medicine 2016; 16(1): 337. Available from: https://doi.org/10.11 86/S12906-016-1323-6

Decroli E. Testosteron and The Benefit for Men's Health. Subbagian Endokrin Metabolik Bagian Ilmu Penyakit Dalam Fak. Kedokteran Unand/ Rsup Dr. M. Djamil Padang 2022.

Khatimah H. Kadar Testosteron Intratestikular Tikus (*Rattus novergicus*) Strain Sprague Dawley Akibat Pemberian Kurkumin Setelah Stimulasi Hcg. Berkala Kedokteran 2015; 11(2): 189-97.

Novita IP. Pemberian Zinc dapat Menghambat Penurunan Jumlah Sel Leydig dan Kadar Testosteron pada Tikus (*Rattus norvegicus*) Jantan Galur Wistar yang Dipapar Asap Rokok Elektrik. Denpasar: FK Universitas Udayana 2021 [Thesis].

Pangkahila W. Tetap Muda, Sehat dan Berkualitas Konsep Anti-Aging Medicine 2017.

Parwata IMOA. Bahan Ajar Antioksidan. Kimia Terapan Program Pascasarjana Universitas Udayana 2016; 4: 1–54.

Permatasari NI. Pemberian Zinc Dapat Menghambat Penurunan Jumlah Sel Leydig Dan Kadar Testosteron Pada Tikus (Rattus Norvegicus) Jantan Galur Wistar Yang Dipapar Asap Rokok Elektrik. Denpasar : FK Udayana 2021. [Thesis]

Radical F *et al*. Aktivitas Penangkat Radikal Bebas dari Batang Pakis (*Alsophila glauca J. Sm*). Majalah Obat Tradisional; 16(3): 156-60.

Roychoudhury S, Chakraborty S, Choudhury AP, Das A, Jha NK., Slama P *et al*. Environmental Factors-Induced Oxidative Stress: Hormonal and Molecular Pathway Disruptions in Hypogonadism and Erectile Dysfunction. Antioxidants 2021; 10(6): 837. Available from: https://doi.Org/10.3390/Antiox10060837

Rusmini H, Fitriani D, Ulfa AM, Gustiawan R. Studi Literatur: Pengaruh Pemberian Ekstrak Daun Bayam Merah (*Amaranthus tricolor L.*) terhadap Indeks Lee dan Massa Lemak Abdominal pada Tikus Putih (*Rattus norvegicus*) Galur Wistar Jantan yang Diberi Diet Tinggi Lemak. Jurnal Ilmu Kedokteran dan Kesehatan 2021; 8(3): 8.

Sathya M. Assaying the Antioxidant Activity of Banana Peel 2014. Available from: https://Scialert.Net/Fulltext/?Doi=Ajbmb.2014.122.129

Syamsuddin SMS, Edy HJ, Supriati HS. Uji Efektivitas Ekstrak Kulit Pisang Goroho (*Musa Acuminate L.*) terhadap Penurunan Kadar Glukosa Darah pada Tikus Putih Jantan Galur Wistar yang Diinduksi Sukrosa. Jurnal Ilmiah Farmasi 2013; 2(1).

Tang Fui M, Dupuis P, Grossmann M. Lowered Testosterone in Male Obesity: Mechanisms, Morbidity and Management. Asian Journal of Andrology 2014; 162: 223. Available from: https://doi.org/10.4103/1008-682x.122365

Wardana NG. Obesitas dan Kadar Testoteron yang Rendah 2016 [Internet] Available from: https://Scholar.Google.Co.Id/Citations?View_Op=View_Citation&Hl=En&User=H66kev4aaaaj&Citation_For_View=H66kev4aaaaj:D1gkvwhdpl0c