The Protective Effect of Physalis angulata L. (Solanaceae) and its Modulatory Action on the Sperms and Testis of Paracetamol Intoxicated Mice

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Abstract

Physalisangulata fruit can be used to rescue fertility levels. Paracetamol is an analgesic but it can be fatal. It can reserve male fertility levels specifically, when a drug is abused as an analgesic but it has other pulchritudinous utilities. Paracetamol is one of those drugs commonly abused as an analgesic but it has fatal tendency which is obvious at high doses. However, its fortuity can be harnessed if administered in therapeutic doses. Otherwise, it can pretermit your life. This study assessed the protective effect of Physalisangulata fruit in rescuing the reproductive performance of laboratory mice exposed to paracetamol (200mg/kg). Male mice aged 23 months of the white Swiss Balb/c strain were divided into 4 groups; the control group that was given normal saline, first group that had alcoholic extract of Physalisangulata fruit (200 mg/kg), while the other one was treated with alcoholic extract of P.angulata fruit and paracetamol (0.50 mg/kg) and the final group was given paracetamol alone (0.50 mg/kg). The result revealed that body weights, organ size for testis, epididymis, seminal vesicles and reproductive indices were significantly reduced as a result of paracetamol exposure. On the other hand, alcoholised fruit extract showed a protective effect on decreased sperm numbers, decreased cellularity, altered testicular histo-morphology following exposure to paracetamol. Seminiferous tubule morphological characteristic was changed to a thicker epithelial cell layer in testes. Hematoxylin-eosin staining technique revealed an improvement in testicular histomorphology after alcoholised fruit extract with a seminiferous tubule morphological characteristic that is somewhat changed to a thicker epithelial cell layer in testes. Paracetamol significantly reduces the body weights and organ size involving testicles, epididymides and seminal vesicles as well as reproductive indices. At the same time, the alcoholic fruit extract showed protective effects on decreased sperm numbers, decreased cell numbers and alteration in reproductive organ histomorphology following paracetamol intoxication. There was an alteration of seminiferous tubule morphological characteristic to a thicker epithelial cell layer in testes. In conclusion, physalisangulata fruit may be useful in rescuing the destructive tendency of paracetamol that is commonly abused as an analgesic but it can reserve male fertility level, when properly administered medically at therapeutic doses. It can equally prevent possible expected complications that may manifest following overdose consequences.

Keywords: Physalisangulata L., Paracetamol, Reproduction, fertility, Herbal, Mice, Sexual behavior.

Introduction

Paracetamol, a daily use antipyretic and analgesic is a common word in all households but usage of the drug has long been discussed due to its toxic nature [1]. Despite its toxicity is not directly fatal like EDTA (Ethylendiamine tetraacetic acid), paracetamol’s toxicity is triggered in overdose or during long term usage conditions [2]. Paracetamol is very toxic to the body. Multiple published researches have known about paracetamol’s impact on reproductive health. Regular intake of entire life causes not only reduced sperm quality but also testicular function damage [3]. Often, daily use of...
high doses of paracetamol also reduces the amount and motility of sperm in humans [4] and continuous usage of a higher dosage for a long period (500-1000 mg/kg) among mice reduces their reproductive ability [5], including reducing their sperm counts and motility in epididymis. Besides, fetal exposure to paracetamol affects the reproduction of children at the ovarian level, where more exposure affects their number of primordial follicles, the body’s follicle formation. It was observed in a reproduction study [6] that exposure to paracetamol in oocytes reduces both primordial follicles and antral follicles in female children. Long-term administration also affects the reproduction, by reducing spermatogenic activity (spermatogenesis and sperm output) and testosterone levels among male offspring [7].

It can be seen from the preceding explanations that toxic substances certain aspects of life and the body. What the collected researches demonstrated, the combined administration proved positive that paracetamol affects fertility in mouse through impairment of sperm characters, ovarian follicles and sexual activity in addition to those Hay-Schmidt et al. revealed. It is noteworthy that oral administration at the rate of 0.05 mg/kg exhibited a significant reduction in serum testosterone level and semen volume within a two-week period. Likewise, extended administration at the same concentration for 42 days notably decreased the sperm count per gram testis weight and percentage motility compared to controls in white mouse adult males. [8, 9]. It undergoes instant liver metabolism through cytochrome P-450 2E1 to form a reactive toxic intermediate, N-acetyl-p-benzoquinoneimine, that depletes hepatic glutathione. Physalisangulata L., a member of the Solanaceae family with so many medicinal merits, has a therapeutic benefit on acute inflammation. Although the evidence on chronic inflammation is presently limited, experiments suggested that it has significant anti-oxidant and anti-inflammatory effects which could help mitigate those harmful outcomes. Plant parts such as seeds, flowers, and fruits have widely been traditionally used in many cultures. Among the traditionally used plants with the therapeutic properties of the Physalisangulata L. fruit are alkaloids, glycosides, terpenoids, saponins, flavonoids, tannins and withanolides contributing to its immunomodulatory, anti-tumor, anti-inflammatory, and antimicrobial effects [10; 11].

In addition to its usage as an immunomodulator and immune-stimulator, it has also been used as an antiparasitic agent against microbes [12; 13]. It could be used in Japan as an antipyretic for various diseases including stomach problems such as ulcers and suppurations such as in boils or wounds (cuts) [14]. In the Brazilian Amazon, it is applied to treat chronic diarrhea [15]. According to studies, the extract leaf of P. angulata L at different doses (250 mg/kg body weight (bw), 500 mg/kg bw, 1000 mg/kg bw, and 1500 mg/kg bw) increases the sperm production and sperm fertility in white mice. Therefore, it can be used to improve human infertility and male with low fetus sperm count [16, 17]. The dosage of 250 mg/kg bw has demonstrated that the testicle malondialdehyde (MDA) level may not increase or may maintain the motile sperm count, which shows an antioxidant property and that the plant could be very useful at this dosage to reduce oxidative stress on sperm quality [14, 18].

The extract had an impact on the intracellular calcium concentration in the sperm which provides a foundation for motility and sperm function [19, 20]. This is an attempt to evaluate the ability of PhysalisangulataL.extract to protect male mice model from infertility caused by paracetamol. It guides how Physalisangulata L. exerts its antioxidative effect on spermatic tissue as well as the testes. It also provides information to see if this organic substance can work as a therapeutic, as a preventive that would keep men’s fertility from being damaged by traditional painkillers. The report will add insights for possible future clinical application, strengthen public health system, which in turn will enhance human reproductive health and solve iatrogenic infertility resulting from drug therapy.

**Material and Methods**

**Plant Extract and Preparation**

Collecting this plant material at: Baghdad, Iraq June 2023 Fruit are identified. At room temperature the fruits shrunk. First drying completely, then grind in electric mill in a fine powder. Later different hot solutions were used to separately soak those. These parts are each dried three times in electric oven at different time. Weighing 50 g of powder plant material tightly before adding 150 mL of 70% concentrated ethanolic solution which later diluted with 30 mL of distilled water (as mentioned earlier). Ethanol infused with crushed plant material poured inside a beaker glass placed over ethanol bath for heating provided. These vials loaded in shaking incubator for some time [21]. To have intensified mixing, agitations are provided to this vials stored in shaking incubator. Filtration performed with filter paper for some time more than final filtrate obtained after removing residue which finally dried on petri dishes inside oven to get extract from powder. Operation of scraping. mg/kg concentration value is finally prepared [22].

**Paracetamol concentrations**

The median which is lethal dose was found this is 0.50mg/kg during present study. Such data is found in literature and effective concentration [23], this was brought from China.

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Experimental design

Therefore, twenty-three male mice aged (6-8) weeks and weighed from (30-35) gram each were collected from Biotechnology Center of Baghdad University. Moreover, these animals were maintained in the animal holdings at College of Education for Girls – Anbar University – Iraq under laboratory animal housing conditions. Scientists care for the mice according to the 1985 National Research Council’s guide for the care and use of laboratory animals as adopted by the Anbar University – College of Science, which entails 12-hour light-dark cycle and a constant temperature maintained at 22°C and environmental condition are controlled. Animals were granted ad-lib access to water and high-quality commercial diet laboratory chow throughout the experimental study. Mice were randomized to five groups that treated by oral gavage of substances over a period of two weeks [24]; therefore, doses administered and concentrations of ingested substances are determined by your previous research [25]. Grouping included is:

G1: After two weeks of intraperitoneal injection with physiological saline.

G2 Mice in the paracetamol group were provided with the 0.65 mg/kg body weight dose of paracetamol twice a day.

G3 Treatment group for mice: A P. angulata extract mixed with Paracetamol.

G4 Plant extract group: The mice received a concentration equaled to 200 mg/ml P. angulata extract. This experimental approach has been conducted to evaluate the effect that Physalisangulata L., extract has on alterations caused by Paracetamol in male reproductive parameters taking into consideration scientific rigours that conform moral standards for animal studies.

Animal Sacrificing and Blood Collection

On the 14th day of the trial at the end of which tissues samples were collected after anaesthetised with diethyl ether and covered by cotton wool by heart puncture from a jugular vein, add 2 ml of blood in micro tube and let it rest inseparable, and centrifuged (3000 rpm) by centrifugal for 10 minutes. After separating the serum from a mixture, they were kept into -20°C to use for the test. After doing so, they were weighed with electronic sensitive scale, then excised the testicles and epididymis (head and tail) by cleaning from any adherent fat and wiped it by filter paper. We investigated the characteristics of sperm for 10 males after randomly tapping testes and epididymis (head and tail) and put it into physiologic saline solution 0.9%, but the others 5 males were disposed of epididymis and testicular (head and tail) because they had been fixed in Bouin’s sections solution that were prepared from 75 ml of saturated aqueous picric acid, 25 ml of 40% formalin and 5 ml of glacial acetic acid for. Even they had preserved into 70% ethyl alcohol solution for seeping the histological section that prepared from the matter was sample [26].

Semen parameters Examination

The testicle was cut into smaller pieces with a sharp scalpel so as to empty the sperm from the seminiferous tubules and then stained with Nigrosin-Eosin stain, afterwards. Specimens were observed under a microscope at 40 x magnification for count of the sperm cells. Afterwards, only dead ones got stained therefore counted percent of live forms [27]. Finally, on the left side of the testicle after been cut off by using a sharp scalpel prepared by adding t10 ml of formalin, 45% to 90% physiological saline solution then we finally counted the sperm that we counted in 80 small squares for cell counting slide they we used the following equation below:

\[
\text{Total number of sperms} = \frac{\text{N}(80)\times4000\times1000\times10)}{10}
\]

The slides prepared this way, containing sperm, were looked through a light microscope and the live and dead sperms were counted. In the second part, we were taught how to classify morphologically abnormal sperms such as variation in head, midpiece, tail and cytoplasmic droplets.

Histological Studies

The right testes of combined control and experimental mice were used for histological examination. They removed these tissues from the mouse; the testicular tissues (wet weight) were wrapped in aluminum foil and preserved in Bouin for 24 hours at room temperature, dehydrated with four changes of xylene and embedded in wax made from paraffin. They obtained the sections (thickness 5 μm) using a rotary microtome, and the tissue sections were stained according to the standard Hazy procedure. The H and E stained the tissue sections, and the observations were reviewed by a research microscope; these images were obtained by a digital camera; the gross appearance was noted as well as the detailed microscopic findings [20].

Statistical Analysis

The data was assessed using SPSS compute utility 16.0 and as per Mann-Whitney analysis; the p ≤ 0.05 level was consider to be significant [28].

Results

Effects on the Studied Weights

Figure 1 clearly revealed the loss of body weight was significantly aged when treated with high doses of paracetamol. Compared with control group. Testicules, epidymal head and tail plus seminal vesicle was significantly increased at 2500mg/kg. and 5000mg/kg group. An extract with a concentration of 200 mg/kg increased conditions and

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parameters of the testes together with epididymal head and tail and the seminal vesicle. And there was the significant difference among groups (P<0.05).

**Effects on Diameters of the Studied Treatments**

P<0.05 decrease in diameter of testes seminiferous tubules and epididymal tubules were 

**Impact on Spermatogenic Cell Percentage and Leydig Cell Numbers**

The table 1, presents the research findings that demonstrate a statistically significant (P<0.05) rise in pro-spermatogonia and primary sperm cells due to dosing with herbal extract and paracetamol, whether combined or separately, in contrast to control and medicated groups. However, the drug itself did not spare sperm precursors, sperm themselves and Leydig’s cells from a P<0.05 fall. Moreover, when treatments administered were only paracetamol compared to a control group there were significant differences (P<0.05) which affected both spermatogenic and Leydig’s cell populations.

**Impact on the Ratio of Healthy to Damaged Sperm**

The result of statistical analysis which is as per below table reveals that Paracetamol injection led to decreasing (P.05) in the percentage of live sperm in testis and epididymal head and tail, whereas percentage of live sperm was significantly increased (P<0.05) after treated with plant extract and control. there is significant difference (P<0.05) in percentage of live sperm between treated group as shown in table 2. The amount of the deformed sperm in epididymis head and tail show an increase (P<0.05) from the table 2, when the male mouses are injected with different dosages of the paracetamol drug. However, this increase shown is non-significant when compared with the dosage that the group which was treated with plant extract or with the control.

**Impact on the Concentration of Testosterone**

Paracetamol injection significantly decreased the serum testosterone levels in mice (P<0.05). However, on contrast to the control group, this concentration rose after administration of extract and it also saved the body from harm by the drug as seen in fig. 3.

**Histological Study**

Histological observation was done on the control treated testicle using a microscope, as shown in Figure 4. Unique changes were seen in the testicular tissue in the treated mice (experimental group) when compared with the control mice. The plant extract treated group had much more organized sperm cells and elongated seminiferous epithelium, a narrow central cavity of the tubules and much tighter interconnected structures with less interstitial spaces. The plant extract treated mice group showed spermatogenesis in different stages as seen in Figure 4a. Disturbing histological changes in the testicular tissue of mice where the combination of plant extract and paracetamol drug were fed was investigated. As seen in Figure 4b, the seminiferous epithelium had a minor disturbance. However, since there were so many sperm presence, it indicates that plant extract may be used as a remedy to compensate for the damages that paracetamol-induced. Histological morphology shown in Figure 4b. In contrast (Figure 4c), the seminiferous tubules were seen with a more vacuous appearance of the epithelial lining, indicating cell death or shedding of cells from the spermatogenic lineages. The reason of interruptions in spermatogenesis and sperm cells death in some tubules is unknown.

**Discussion**

There are many other possible variables that can explain why the bodyweights of the treated groups declined after the injection of paracetamol. It could be because the drug induced some problems in the digestion system of the males, and this, in turn, restricted their appetite and thus palate diminution, but we think paracetamol has done more thing than that because there are researches that claimed a high elevation in paracetamol around the upper gastrointestinal tract increases the chances of disorders concerning the tip of the gut and even caused a terribly lethal gastric bleed. The experiment we conducted with these male mice has shown its culpability in their weight [29; 30]. Other sources have claimed that paracetamol makes the reproductive ability of male Caucasian rats to be inhibited if they are treated with it. It can be because of some modifications in histology of testes in relation to the formation of NAPQI [8]. It could be really as a result of the palate again because of a shift in histological composition of something else caused by this paracetamol [31].

This effect can largely be stressed on their males since their weights significantly came down. High doses of paracetamol can lead to shrinkage of the Uterus; Ovaries, female; Testis, [32]. Another study claims that the administration of paracetamol resulted in endocrine disruptors of the gonadotropins on biosynthetic pathways. Testosterone is the major driver of the development of the seminal vesicles, the vas deferens and the epididymis during the embryogenesis of a human being. On the other hand, dihydrotestosterone is the main inducer of the development of the testis, the scrotum and the penis and other structures [33]. Besides, testosterone plays a great role in the structural and histological features and subsequent functionality [34].
Moreover, the effect of the medicinal compound in conjunction with drugs on the mass of organs motility in male rats [35; 36]. As for histological experiments, drugs and particularly androgens such as testosterone can induce drug-induced metabolic disorder. This is one of the major disorders that have a significant as hydrogen peroxide (H2O2) and superoxide anions (O2-) from the P-450-dependent enzyme system can play a role in the maintenance of oxidative stress, which results in the creation of free radicals. Ultimately, scientific evidence has revealed that using medicinal plants improves the performance of sexual glands, thereby generating motile and viable spermatozoa that are endowed with improved tissue architecture [36, 37].

Paracetamol can be accumulated in the body and form cumulative concentrations, which potentially induce oxidative stress and ultimately cause tissue damage in testis. Additionally, oxidation processes resulting in lipid peroxidation affect the lipid matrix of sperm, which weakens the prevalence of membrane structures needed for the survival of these reproductive cells. As a result, they might undergo non-vital sperm or have an abnormal and defective formation of midpiece’s morphology structure. According to Hseu [38], the impact of paracetamol can be so dramatic in severe cases that it results in the complete termination of the spermiogenesis process. Tahvilzadah [39] and Vesal [40] proved that the oral administration of paracetamol to mice led to a histological assessment of reduced sperm cell counts. The observed effect is due to reduced survival rates associated with the diminished frequency of sperm quantity in each ejaculation. The P. angulata’s plant extracts should preserve histology cells in a good state as it is proved there are an abundance of organic compounds that promote tissue regeneration and inhibit oxidative processes [41, 42, 43].

Conclusion
The final results of the research reveal that the use of extract of Physalisangulata L. has proven to be effective in preventing the harmful impacts of paracetamol on the male reproductive system of mice, leading to the reduction in either body and testicular weight, reduced sperm counts and altered sperm morphology, while the administration of P. angulata extract has prevented it and maintained their normal histology, in addition to improving both sperm counts and quality. Conclusion: Considering the harmful effects of paracetamol, which is a widely used analgesic for treating several disorders, and its association with male fertility reduction, the use of Physalisangulata fruits has become important to protect male fertility against their adverse effects.

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The authors declare that the present study has no financial issues to disclose.

Conflict of interest
None

Authors contributions
Manuscript revision was contributed by all the authors, and they approved the submitted version.

Ethical Statements
Besides, the experimental protocol was approved by a Scientific Research Ethics Committee at Anbar University (Anbar University), to maintain ethical guidelines for research investigations, with (Approval No, 35/2023).

Fig. 1. The figures above reveals that the extract of P.angulata is significantly protecting the weights of epididymis tail of epididymis and seminal vesicle (g).* the the level (P<0.05). The blanks are controlled with saline solution, Paracetamol 0.65 mg /kg and Extract =P.angulata 200mg/kg.(LSD5%=2.233).
Fig. 2. The diameters of seminiferous tubules (µM), epithelial cells in the epididymal head (µM), and the height of tubules in the epididymis (µM) were protectively affected (LSD5%=1.076).

TABLE 1. A protective impact on Leydig cell number and percentage of cells originating from the testis's seminiferous tubules

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Prosperms%</th>
<th>Primary sperm cells</th>
<th>%spermatids</th>
<th>%Sperm</th>
<th>Leydig cell Preperation</th>
<th>Transactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>12</td>
<td>12.86</td>
<td>44.46</td>
<td>34.68</td>
<td>11.46</td>
<td>G1</td>
</tr>
<tr>
<td></td>
<td>14.6</td>
<td>14.73</td>
<td>41.2</td>
<td>31.47</td>
<td>9.92</td>
<td>G4</td>
</tr>
<tr>
<td></td>
<td>15.66</td>
<td>15.13</td>
<td>43.06</td>
<td>31.15</td>
<td>13.44</td>
<td>G3</td>
</tr>
<tr>
<td></td>
<td>16.13</td>
<td>14.2</td>
<td>35.06</td>
<td>29.61</td>
<td>6.33</td>
<td>G2</td>
</tr>
<tr>
<td></td>
<td>0.12</td>
<td>0.961</td>
<td>2.23</td>
<td>1.54</td>
<td>1.076</td>
<td>LSD 5%</td>
</tr>
</tbody>
</table>

TABLE 2. The effectiveness of prophylaxis in reducing the number of abnormal and viable sperm in the testes and epididymis (%)

<table>
<thead>
<tr>
<th>Head of epididymis</th>
<th>Tail of epididymis</th>
<th>%Testicle</th>
<th>Head of epididymis</th>
<th>Tail of epididymis</th>
<th>%</th>
<th>Transactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.2</td>
<td>7.86</td>
<td>96.93</td>
<td>97.06</td>
<td>97.26</td>
<td>97.26</td>
<td>G1</td>
</tr>
<tr>
<td>8.46</td>
<td>7.33</td>
<td>97.66</td>
<td>95</td>
<td>94.13</td>
<td>94.13</td>
<td>G4</td>
</tr>
<tr>
<td>8.26</td>
<td>8.93</td>
<td>95.26</td>
<td>94.13</td>
<td>92.2</td>
<td>92.2</td>
<td>G3</td>
</tr>
<tr>
<td>14.61</td>
<td>16.46</td>
<td>*81.86</td>
<td>82.5</td>
<td>*82.6</td>
<td>*82.6</td>
<td>G2</td>
</tr>
<tr>
<td><strong>2.65</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LSD 5%</td>
</tr>
</tbody>
</table>

Fig. 3. The LSD5 percent of 0.236 has a protective action against the level of lipotropic hormone in the testicles

* indicates significant difference
Fig. 4. A, An increase in seminiferous tubules diameter and interstitial cells has been observed upon 200 mg/kg plant extract treatment (H&E 10x). B, treated with 200 mg/kg plant extract and with 0.56 mg/kg paracetamol demonstrate increased density of interstitial cells. Increase in interstitial cells positively correlated with duration and intensity of spermatogenesis (H&E 10x). Degree of spermatogenesis increased by treatment with 200 mg/kg plant extract and 0.56 mg/kg paracetamol and was accompanied by increase in diameter and thickness of tubules and epithelial cells respectively (H&E 40x). C, Treatment consisting of treatment with paracetamol alone animals were observed degeneration, sperm deficiency and reduced thickness of epithelial cells (H&E 400).

References


التأثير الوقائي لنبات Physalis angulate L. (Solanaceae) في إنقاذ الأداء التناسلي للمختبر الفئران عند تناول جرعات عالية. مع ذلك، يمكن تسخير مفعوله إذا تم تناوله بجرعات علاجية. وإلا فإنه يمكن أن يؤخر تطور الفيوفيت.

الخلاصة:
يمكن استخدام فاكهة Physalisangulata لرفع مستويات حيوانات المنوية. الباراسيتامول مسكن ولد يحافظ على مستويات خصوبة الرجال، مع ذكر أن باراسيتامول يقلل بشكل كبير من أوزان الجسم وحجم الأعضاء بما في ذلك الخصيتين والبربخ. ومع ذلك، يمكن تحسين نتائج الفيوفيت بعد تناول الكحول. الحيوانات المنوية والخصية في الفئران المسكرة بالباراسيتامول. يمكن استخدام الفاكهة لتحسين نتائج الفيوفيت بعد تناول الكحول. 

المستخلص:
النى هاشم مصلح, زينة هاشم مصلح
العنوان: التأثير الوقائي على Physalisangulata L. (Solanaceae) في إنقاذ الأداء التناسلي للمختبر الفئران عند تناول جرعات عالية.

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يمكن استخدام فاكهة Physalisangulata L. (Solanaceae) لرفع مستويات حيوانات المنوية. الباراسيتامول مسكن ولد يحافظ على مستويات خصوبة الرجال، مع ذكر أن باراسيتامول يقلل بشكل كبير من أوزان الجسم وحجم الأعضاء بما في ذلك الخصيتين والبربخ. ومع ذلك، يمكن تحسين نتائج الفيوفيت بعد تناول الكحول. الحيوانات المنوية والخصية في الفئران المسكرة بالباراسيتامول. يمكن استخدام الفاكهة لتحسين نتائج الفيوفيت بعد تناول الكحول. 

الخلاصة:
يعتبر فاكهة Physalis angulate L. (Solanaceae) مفيدة في إنقاذ الأداء التناسلي للمختبر الفئران عند تناول جرعات عالية. مع ذلك، يمكن تسخير مفعوله إذا تم تناوله بجرعات علاجية. وإلا فإنه يمكن أن يؤخر تطور الفيوفيت.