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Genetic, Biochemical and Bacteriological Study on Coconut Water

A thesis

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Anssam

Summary

As coconut water is of great importance in different biotechnological and biological applications, this study involved three main axes which can be represented by genetic, biochemical and bacteriological applications to study the biological characteristics of coconut water and its future applications.

Genetic study included studying the ability of coconut water as a suitable medium for the merging of foreign DNA molecules through recombination process and expressing their genes in the new host. The plasmid pEGFP was used as a proof for the completion of binding process and screening the hybrid fluorescent proteins which are coded by this plasmid.

The plasmid pEGFP was added to fresh coconut water at different concentrations (0.1µg/ml, 0.081µg/ml, 0.06µg/ml,0.04µg/ml and 0.02µg/ml) in order to detect its ability to merge with DNA from coconut water to examine the ability of water to synthesize recombinant proteins. Results showed that 0.1µg/ml gave positive green color after 4hrs and 5hrs.

Biochemical application involved studying the effect of coconut water on liver metabolism *in vivo* white laboratory female mice were used for this experiment, they were injected intrapretonially with different concentrations of coconut water for different periods of time.

Results revealed that coconut water had no significant effect on the levels of GPT, GOT and ALP in female mice when compared to control groups treated with normal saline.

The study also involved detecting the possibility of using coconut water as alternative culture medium for growing pathogenic and non pathogenic bacteria

isolates and comparing it with traditional culture media. Results showed that coconut water at different concentrations between 10-100% was better culture media in comparison to any culture medium (Nutrient Agar, Brain Heart Infusion Agar, MacConkey Agar, Gauza Agar) depending on the viable bacterial count.

Also the sensitivity of pathogenic and non pathogenic bacteria against antibiotics (Chloramphenicol, Penicillin, Gentamycin, Erythromycin, Lincomycin, Trimetheprim, Ciproflaxin and Streptomycin) was tested on coconut water agar culture medium at different concentrations between 10-100% and results were compared with Muller-Hinton agar medium.

Results showed that the sensitivity of bacteria against the antibiotics was higher on Muller-Hinton agar medium.

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Abbreviations	*Words*
GOT	Glutamate Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase
ALP	Alkaline Phosphatase
PM	Particulate Matter
BHI	Brain Heart Infusion agar
S-S agar	*Salmonella-Shigella* agar
MRS	Man Rogosa Sharp
TG	Triglyceride
HDL-C	High Density Lipoprotein
VLDL	Very Low Density Lipoprotein
LDL	Low Density Lipoprotein
CNO	Coconut Oil
CNW	Coconut Water
NA	Not analyzed

1.1 Introduction

The coconut palm (*Cocos nucifera*) is considered the "tree of life" and the coconut is spoken of with great reverence because of its cultural significance, economic importance and different uses. The coconut palm is truly a remarkable plant because it provides wood, materials for shelter and handicrafts, refreshing drinks, food, animal feed and cash from copra. It has been said that there are 121 uses of the coconut palm.

These uses did not include ecological functions such as shade, protection from wind, sand and salt spray, erosion and flood control, coastal reclamation, animal and plant habitat, and soil improvement. Coconut thrives in the alkaline coral sands of the atolls and the fertile volcanic soils of larger islands (Osborn and Aarlbersberg, 2003).

The coconut is a versatile economic crop. Its many uses encompass food, energy, industrial and even construction applications. The fruit of the coconut palm is the main source of many food products foremost among them being coconut oil (CNO). It is derived from the meat or solid endosperm. Inside the shell is the coconut water (CNW) or liquid endosperm (Banzon *et al.*, 1990).

Coconut is the healthiest food/medicine you can put inside your body. Coconuts have more electrolytes than any other fruit, vegetable or water on Earth. Coconut is a miraculous food/medicine. Coconuts have, and to this day still do, saved many lives all over the world (for hundreds of years). Coconut water is the same as human blood plasma, and has been used as plasma for blood transfusions, etc in many countries and islands throughout the world (Allia, 2004).

Coconut water is the liquid endosperm inside young coconuts. As the coconut matures this liquid largely becomes absorbed into the flesh found in mature coconuts. Coconut water has long been a popular drink in the tropics, especially in Tropical Asia and Trinidad and Tobago, where it is available fresh or bottled. It is naturally fat-free and low in food energy (16.7 calories or 70 kJ per 100 g). Due to its sterility, pH, mineral, and sugar content, coconut water had been successfully used as liquid in intravenous therapy emergency situations (Campbell-Falck *et al*, 2000).

Aims of the study:

1- Determination of the capability of coconut water to facililate expression of fluorescent genes in pEGFP plasmid.

2- Determination of the efficiency of coconut water as an alternative growth medium for pathogenic and nonpathogenic bacteria and to test its ability to reduce antibiotic resistance of pathogenic isolates.

3- In *vivo* study to determine the effect of coconut water on liver functions.

1.2 Literature Review

1.2.1 Coconut Palm

The coconut palm (*Cocos nucifera*) is considered the tree of life, since it is one humanity part of this plant can be utilized: roots, husk, leaves, inflorescence and fruit ((Bourdeix *et al.*, 2005).

The coconut palm is found throughout the tropics, where it is interwoven into the lives of the local people. It is particularly important in the low islands of the Pacific where, in the absence of land-based natural resources, it provides almost all the necessities of life—food, drink, oil, medicine, fiber, timber, thatch, mats, fuel, and domestic utensils.

For good reason, it has been called the "tree of heaven" and "tree of life." Today it remains an important economic and subsistence crop in many small Pacific island states (Bourdeix *et al.*, 2005).

1.2.1.1 Distribution

A- Native range (Clarke and Thaman, 1993)

Coconut is native to coastal areas (the littoral zone) of Southeast Asia (Malaysia, Indonesia, Philippines) and Melanesia. In prehistoric times wild forms (niu kafa) are believed to have been carried eastward on ocean currents to the tropical Pacific islands (Melanesia, Polynesia, and Micronesia) and westward to coastal India, SriLanka, East Africa, and tropical islands (e.g., Seychelles, Andaman, Mauritius) in the Indian Ocean.

In these regions, the palms were able to establish themselves on sandy and coralline coasts. Coconut is either an introduction or possibly native to the Pacific coast of Central America.

B- Current distribution

The coconut palm has wide pantropical distribution. It is a ubiquitous sight in all tropical and subtropical regions 23° north and south of the equator.

It is also found outside these latitudes, where it will lower, but fruits fail to develop normally (Rehm and Espig, 1991).

It is believed that Polynesians migrating into the Pacific 4500 years ago brought with them aboriginal selections (niu vai).

At about the same time, people from Indo-Malaya were colonizing the islands of Micronesia. Malay and Arab traders spread improved coconut types west to India, SriLanka, and East Africa about 3000 years ago.

Coconuts were introduced into West Africa and the Caribbean (including the Atlantic coast of Central America) during the 16th Century by European explorers (Handy, 1985).

Through the involvement of people, the palm spread in land and is now grown over a wide variety of soil types and up to an altitude of 600 m (1970 ft) at the equator.

It is an important plant in the lives and economies of people in the following countries (Lamb, 1987):

- **Southeast Asia** Burm, Indonesia, Malaysia, Philippines,Singapore, South China (Hainan), hailand, Vietnam.
- **Indian Subcontinent, Indian Ocean** Bangladesh, South India, SriLanka, and islands of Andaman, Nicobar, Seychelles.

1.2.1.2 Botanical Description

The following description applies to the all variety of coconuts, which is the dominant type grown the world over (Whistler, 2000; Harries, 1978) as shown in figure (1-1).

- Preferred scientific name: *Cocos nucifera* L.
- There are no other known species in the genus *Cocos*.
- Family: *Arecaceae* (palm family)
- Subfamily: *Cocoideae*
- Non-preferred scientific names *Palma cocos* Miller
- Common names: coconut, coconut palm (English)

1.2.1.2.1 Size (Whistler, 2000)

A crown of fronds is borne on a single unbranched stem with aerial growth from a single growing point. A 40-yearold palm typically attains a height of 20–22 m (66–72 ft), and an 80-year-old palm may attain a height of 35–40m (115–130 ft). The canopy has a diameter of 8–9 m (26–30 ft).

1.2.1.2.2 Form (Whistler, 2000)

The fronds in a mature healthy palm describe a sphere and are evenly distributed in all directions from the growing tip. In heavily bearing palms, the weight of nuts may push down on the horizontal fronds, resulting in an X-shapedcanopy in which no fronds are held in a near-horizontal position.

1.2.1.2.3 Flowers (Whistler, 2000)

The coconut palm is monoecious, i.e., with male and female flowers on the same inflorescence, called a spadix, that develops within a woody sheathe or spathe. At flowering, the spathe splits lengthwise to expose the spadix. Each spadix consists of a main axis 1–1.5 m (3.3–5 ft) in length with 40–60 branches or spikelets bearing the flowers. Each spikelet carries from zero to three female flowers ("buttons") at its base and several hundred male flowers above. Thus a spadix will have several thousand male flowers but only 40–60 buttons.

The male flower has sixperianth segments surrounding six stamens. The larger female flowers are globose and consist of six perianth segments in two whorls, a tricarpellate ovary and trifid stigma. Following pollination, only one carpel develops into the seed, the other two aborting.

The perianth persists at the base of the mature fruit.Anthesis is usually completed before the female flowers are receptive, encouraging cross-pollination. However, pollination can occur between flowers of successive spadices on the same palm. Under favorable growing conditions, first flowering occurs about 4–5 years after planting.

Figure (1-1): Coconut Palm (Whistler, 2000).

1.2.1.3 Fruit Description

The fruit is a fibrous drupe. It consists of, from the outside in, a thin hard skin (exocarp), a thicker layer of ibrous mesocarp (husk), the hard endocarp (shell), the white endosperm (kernel), and a large cavity filled with liquid (Water)(Ohler and Magat, 2005).

When immature, the exocarp is usually green, sometimes bronze. Wide variation in fruit shape and size exist within types and populations. Fruit shapes vary from elongated to almost spherical and weigh between 850 and 3700g when mature (Ohler and Magat, 2005).

Figure (1-2): *Cocos nucifera* (L.) (Ohler and Magat, 2005).

1.2.1.4 A little More Coconut Water History

The English name coconut, first mentioned in English print in 1555, comes from Spanish and Portugese word coco, which means "monkey face."

Coconuts are the fruit of the coconut palm, botanically known as cocos nucifera, with nucifera meaning "nut-bearing." The fruit-bearing palms are native to Malaysia, Polynesia and southern Asia, and are now also prolific in South America, India, the Pacific Islands, Hawaii and Florid (Jeganathan, 1992).

The light, fibrous husk allowed it to easily drift on the oceans to other areas to propagate. In Sanskrit, the coconut palm is known as kalpa vriksha, meaning "tree which gives all that is necessary for living," since nearly all parts of the tree can be used in some manner or another. The coconut itself has many food uses, including milk, meat, sugar and oil as well as functioning as its own dish and cup. The husk was also burned for fuel by natives, but today a seed fiber called coir is taken from the husk and used to make brushes, mats, fishnets, and rope. A very potent fermented toddy or drink is also made from the coconut palm's sap (Wagner *et al.*, 2002).

Coconut oil, a saturated fat made from dried coconut meat, is used for commercial frying and in candies and margarines, as well as in non-edible products such as soaps and cosmetics (Leong and Shui, 2002; De Sousa *et al.*, 2005).

1.2.2 Coconut Water

Coconut water is the juice of the endosperm found within the cavity of the coconut, which begin to form around 2 months after the natural opening of the inflorescence ((Bourdeix *et al.*, 2005).

The best time to collect the fruit is at the sixth month of growing, when the maximum volume of water is reached, corresponding to ~25%

of the fruit weight. A fully mature coconut contains about 250ml of water while the less mature ones have considerably more, around 470ml (Simpkins *et al.*, 2000).

Hawaiians call coconut water *noelani* (no-way lah-nee), which means "dew from the heavens." Coconut water has a long history of use as both a food and as a medicine. Although a variety of fruits grow in abundance in many tropical locations, coconut water is prized above all other juices. It not only satisfies thirst but invigorates the body and brings about a sense of well-being and renewed health. Women are encouraged to drink it when pregnant and nursing so their milk will provide all the nutrients necessary for a healthy baby. The first food an infant receives as it begins weaning is coconut water and coconut jelly (soft immature coconut meat) (Oliveira *et al.*, 2005).

1.2.2.1 Coconut Water Composition

Coconut water contains a variety of nutrients including vitamins, minerals, antioxidants, amino acids, enzymes, growth factors, and other nutrients (Simpkins *et al.*, 2000).

Coconut water is a good source of the major minerals like magnesium, calcium, and potassium. It is particularly rich in potassium, an essential nutrient; one 8-ounce cup of coconut water has more potassium than a banana (Nadanasabapathy and Kumar, 1999).

It also contains a variety of trace elements such as zinc, selenium, iodine, sulfur, manganese, boron, molybdenum, and others (Oliveira *et al.*, 2005).

These are derived from volcanic soils and seawater from which the coconut palms are grown. All of these minerals are in the form of

electrolytes so they are easily absorbable by the human body (Campos *et a.l*, 1996).

Many of the health benefits attributed to coconut water can be traced to its mineral content. Coconut water is relatively low in sodium (Sousa *et al.*, 2005).

The basic composition of coconut water is 95.5% water, 4% carbohydrates, 0.1% fat, 0.02% calcium, 0.01% phosphorous, 0.5% iron, in addition to amino acids, vitamin C, B complex vitamins and mineral salts (Robards and Antolovich, 1995).

The nutritional composition of coconut water obtained from fruits at different stages of maturity has been determined. The medium is rich in proteins, amino acid sugars, vitamins, minerals and growth hormones essential to the promotion of tissue growth (Tulecke, 1991), as it is shown in tables (1-1, 1-2).

Table (1-1): Vitamins, Growth factor, Sugar alcohol and Mineral of Coconut Water (Tulecke, 1991).

Compound	Concentration (mg/100ml)
Potassium	**312.0**
Chloride	**183.0**
Sodium	**105.0**
Phosphorus	**37.0**
Magnesium	**30.0**
Sulfur	**24.0**
Iron	**0.10**
Copper	**0.04**

Table 1-2: Vitamins, Growth factor, Sugar alcohol and Mineral of Coconut Water (Tulecke, 1991).

Compound	Concentration (mg/l)
Nicotinic acid	**0.64**
Pantothenic acid	**0.52**
Biotin	**0.02**
Riboflavin	**0.01**
Folic acid	**0.003**
Thiamine	**Trace**
Pyridoxine	**Trace**
Auxin	**0.07**
Gibberellin	**Not determined**
1,3-Diphenylurea	**5.8**
Sorbitol	**15.0**
M-inositol	**0.01**
Scyllo-inositol	**0.05**

Major chemical constituents are sugars and minerals while fat and nitrogenous substances from a minor fraction (Snowdon and Schultz, 2002) and as it was shown tables (1-3, 1-4).

Table (1-3): Nutrient content of a variety of coconut foods (per 100 gram edible portion) (Snowdon and Schultz, 2002).

Nutrient Content	Water [immature]	Water [mature]
Energy [kcal]	**16**	**22**
Moisturariety [g]	**97.0**	**92**
Protien [g]	**NA**	**0.3**
Fat [g]	**NA**	**0.2**
Sugars [g]	**4.1**	**5**
Dietary Fibre [g]	**0**	**0**
Potassium [mg]	**NA**	**310**
Iron [mg]	**NA**	**1.1**
Vitamin C [mg]	**NA**	**2**

NA= Not Analyzed

Table (1-4): Content of nutrients in an average-sized coconut (Snowdon and Schultz, 2002).

Nutrient Content	Water [immature]	Water [mature]
Average- size Coconut weight[g]	**400**	**300**
Energy [kcal]	**64**	**66**
Moisture [g]	**388**	**276**
Protien [g]	**NA**	**0.9**
Fat [g]	**NA**	**0.6**
Sugars [g]	**16.4**	**15**
Dietary Fibre [g]	**0**	**0**
Potassium [mg]	**NA**	**930**
Iron [mg]	**NA**	**3.3**
Vitamin C [mg]	**NA**	**6**

NA|= Not Analyzed

1.2.2.2 Properties of Coconut Water

Coconut water has several properties such as:

A- Medicinal and Therapeutical Properties

Coconut water has a therapeutic effect on the urinary and reproductive systems. It is reported to clear-up bladder infections, remove kidney stones, and improve sexual vitality (Macalalag and Macalalag, 1987).

Coconut water is good for infants suffering from intestinal disturbances, oral dehydration medium, contains organic compound possessing growth promoting properties, keeps the body cool(Campos *et al.*, 1996).

Effective in treatment of kidney and urethral stones, can be injected intravenously in emergency case. Found as blood plasma substitute because it is sterile, does not produce heat, does not destroy red blood cells and is readily, accepted by the body, aids and quick absorption of the drug and makes their peak concentration in the blood easier by its electrolytic effect, urinary antiseptic and eliminates poisons in case of mineral poisoning. An anti dotes to ward off the ill effects of tobacco and alcohol (Macalalag and Macalalag, 1987).

In addition, if has been advised to gastric disturbs treatment, inhibition of vomit caused by cholera, treatment of dysentery and for infant feeding (Santoso *et al.*, 1996; Pummer *et al.*, 2001).

B-Clinical and Medicinal Properties (Santoso *et al.*, 1996; Fagundes Neto *et al.*, 1993; Wharta *et al.*, 2004).

- Cardioprotective: helps regular blood pressure (due to high potassium); improves circulation.
- Reduces swelling in hands and feet.

- Prevents abnormal blood clotting.
- Helps balance blood sugar in diabetics.
- Improves digestion.
- Reported by some people to reverse cataracts.
- Contains nutrients that feed friendly gut bacteria.
- Helps relieve constipation or diarrhea.
- Possesses anti-aging properties.
- Nutritional support for healthy skin: restores strength and elasticity, to skin; reduces age spots; reduces wrinkles and sagging.
- Regulates the functioning of the intestine which promotes smoother, more hydrated skin.
- Enhances healing of wounds and lesions.
- Supports good vision and provides nutritional support in those who have a tendency towards glaucoma.
- Contains potent antioxidants.
- Nutritionally supports immune function.
- Provides nutrients important in preventing osteoporosis.

C- Healthy Growth Promoting Properties

Coconut water contains organic compounds possessing healthy growth promoting properties that have been known to help (Petroianu *et al.*, 2004; Sylianco *et al*., 1992; Khan *et al*., 2003).

- Keep the body cool and at the proper temperature.
- Orally re-hydrate your body; it is an all natural isotonic beverage.
- Carry nutrients and oxygen to cells.
- Naturally replenish your body's fluids after exercising.

- Raise your metabolism.
- Promote weight loss.
- Boost your immune system.
- Detoxify and fight viruses.
- Cleanse your digestive tract.
- Control diabetes.
- Aid your body in fighting viruses that cause the flu, herpes, and AIDS.
- Balance your pH and reduce risk of cancer.
- Treat kidney and urethral stones.
- Boost poor circulation.

1.2.2.3 Application Coconut Water on the Body (Andrade *et al.*, 2002).

- Prevents prickly heat and summer boils and subsides the rashes caused by small pox, chicken pox, and measles.
- Kills intestinal worms.
- Presence of saline and albumin makes it a good drink cholera cases.
- Checks urinary infections.
- Excellent tonic for the old and sick.
- Cures malnourish.
- Diuretic**.**
- Preservation of sheep preantral follicles *in situ.*

1.2.2.4 Coconut Water –an Ideal Drink

The clear liquid in the interior of a coconut is commonly referred to as "coconut water." It is a refreshing and cool, acclaimed by many to be the "perfect drink ".In a healthy, undamaged coconut, the water is sterile.

Its sodium and potassium content makes it an ideal drink for rehydration (Adams and Bratt, 1992).

During World War II, coconut water was used intravenously to treat patients suffering from blood loss when blood plasma was not available (Petroianu *et al.*, 2004).

It is a ready source of clean drinking water, especially after a natural disaster (cyclones, looding) (Jackson *et al.*, 2004).

Characteristics of the water change as the coconut ages. A very young coconut (about 3–5 months, before the endosperm begins to form) has tasteless water that is somewhat as stringent. Water from a mature coconut is slightly salty to the taste, although for coconuts grown well inland, the salty taste disappears. The best time to harvest a coconut for drinking is at age 6–7 months, just as the jelly-like endosperm begins to form. At this stage the water has maximum sweetness and low acidity. Nuts harvested at this age can be stored only 2–3 days before the water begins to sour (Salim *et al.*, 2002).

1.2.2.5 Coconut Water Facts (Khan *et al.*, 2003).

- Coconut water is more nutritious than whole milk - Less fat and No Cholesterol!
- Coconut water is healthier than orange juice - much lower calories.

- Coconut water is better than processed baby milk- It contains lauric acid, which is present in human mother's milk.
- Coconut water is naturally sterile - water permeates though the filtering husk!
- Coconut water is a universal donor- It's identical to human blood plasma.
- Coconut water is a natural isotonic beverage - the same level we have in our blood.
- Coconut water is very high in Chloride at 118mg, compared to others drinks.

1.2.2.6 Coconut Water May Play Significant Role on Body

A- Heart Disease

Coconut water is known as a heart tonic and is used to strengthen the heart and improve circulation. Research bears this out. Animal studies show that coconut water consumption reduces plaque formation in arteries, thus reducing risk of heart attack and stroke.

High blood pressure is one of the primary risk factors associated with heart disease and stroke. The minerals potassium and magnesium are known to help reduce high blood pressure. Human studies show that coconut water, which is a good source of both of these minerals, is effective in reducing high blood pressure and increasing circulation (Alleyne *et al*., 2005).

Studies also demonstrate that coconut water consumption reduces the risk of heart failure in heart disease patients (Shah *et al*., 1996).

B- Diabetic Disease

Due to coconut water improves blood circulation; it is of benefit to diabetics. Coconut water helps dilate blood vessels, improves blood flow, and reduces plaque formation.

Coconut water also contains certain forms of dietary fiber and amino acids that help moderate sugar absorption and improve insulin sensitivity (Santoso *et al.*, 1996).

C- Heatstroke and Dehydration Problems

In the tropics where heatstroke and dehydration are common problems, coconut water is far more effective in relieving symptoms associated with these conditions than either plain water or fruit juice.

Over the past two decades coconut water has been used extensively as a treatment for cholera, dysentery, influenza, and other infectious diseases that promote dehydration.

Coconut water has saved thousands of lives, especially of children in underdeveloped countries. (Santoso *et al.*, 1996).

Ordinary water is not effective in treating severe dehydration caused by vomiting and diarrhea, but because of coconut water's unique chemical composition it is able to rehydrate the body and give it the strength and energy it needs to fight off the infection. Death rates from cholera are high, but with the use of coconut water survival rates increase to 97 percent (Fagundes *et al.*, 1993).

1.2.2.7 Benefits of Coconut Water

Some of the most interesting components of coconut water are the plant growth hormones, particularly cytokinins. Cytokinins are a group of hormones that regulate growth, development, and aging. In some respects they are similar to human hormones with a similar name of cytokines.Cytokinins are also known as anti-aging hormones. Cytokinins regulate cell division and influence the rate at which plants age. Depending on the amount of cytokinins present, the aging process in plants can be either accelerated or retarded (Kobayashi, 1995).

One of the active sites of cytokinin production is in the roots. From here the hormone is carried by the sap throughout the plant—much like our bloodstream disperses hormones. Portions of plants that are deprived of cytokinins age faster than normal, conversely, if additional cytokinins are added to a plant, normal aging is retarded. Cytokinins also have an anti-aging effect on human cells and tissues. Normal human cells, as they age, go through a progressive and irreversible accumulation of changes until they reach a stage at which they finally die. Young cells are plump, round, and smooth. As they age they become irregular in shape, flatten out, enlarge, and fill up with debris; cell division slows down and eventually stops, which is ultimately followed by death. When cytokinins are added to the culture medium, cells don't act their age. The normal sequence of aging slows down considerably. Cells do not undergo the severe degenerative changes that ordinarily occur (Rattan and Clark, 1994).

Although the total lifespan of human cells is not increased much, the cells remain significantly more youthful and functional throughout their lifetime. For example, treated cells after they have reached the final stage

of their lifespan and no longer divide, look and function like untreated cells half their age. Treated cells never undergo the severe degenerative changes experienced by untreated cells. In all respects, their youth is extended into old age. Because of their effects on human cells, cytokinins have been tested for the possible treatment for age spots, wrinkles, sagging, and dry skin. One of the factors that cause wrinkles and sagging skin is the aging and breakdown of connective tissues in the skin. Connective tissues give the skin strength and elasticity. When cytokinins are applied to the skin, they stimulate cell division of connective tissue which replaces older, damaged tissue with functionally younger tissue. The result is that on the surface of the skin, wrinkles tend to flatten out. Dry, aging skin is replaced with smoother, softer skin. This has led some cosmetic manufacturers to add cytokinins to facial creams and lotions. Coconut water is the richest natural dietary source of cytokinins. Researchers have suggested the possibility that consuming a rich source of cytokinins, such as coconut water, may produce an anti-aging effect on the body, reducing risk of developing degenerative and age related diseases. In regulating cell growth, cytokinins also prevent mistakes that may lead to the development of cancer. Normal cells are kept healthy while cancerous cells are programmed to die, preventing them from growing and spreading. Subsequently, the anti-cancer effects of cytokinins have been well documented (Adair and Brennan, 1986; Dolezal, 2006).

1.2.2.7 Important Coconut Water for Plant

Coconut water is often used as a supplement in media for the growth of plant tissue cultures. The endosperm nourishes the growing plant and abounds in proteins, amino acids, sugars, vitamins, minerals and growth hormones that are essential to promote tissue growth. (Tulecke, 1991; Kobayashi, 1995).

This nutrient-rich liquid endosperm of green coconuts is freeze-dried using a special process that preserves the activity of nutrients, to produce cococin. The greatest amount of coconut water is found in young, green coconuts and provides nourishment for the growth of the solid endosperm (coconut meat) inside the hard shell of the fruit. When the fruit matures, both the solid endosperm and the remaining coconut water serve as nutrients for the developing embryo and seedling. Thus coconut water serves as a natural reservoir of nutrients to promote tissue growth (Tulecke, 1991).

1.2.2.8 Coconut Water Uses

In some countries coconut water is used as a solution for oral hydration, as part of the daily diet and as a protein supplement when nutritional deficits are intense. During the Second World War, coconut water was even used instead of saline solution during emergency surgeries (Campbell-Falck, 2000).

Some studies suggested that coconut water can be used for intravenous rehydration (Campbell-Falck, 2000).

Other studies suggest that coconut water can be used for electrolyte replacement in a wide range of situations (Chavalittamrong *et al.*, 1982).

Studies have compared the chemical composition of coconut water with teas (Collares and Souza, 1985).

Still soft drinks (Chavalittamrong *et al.*, 1982). Carbonated soft drinks (Collares and Souza, 1985).

Isotonic drinks and oral rehydration solution (ORS) (Adams and Bratt, 1992).

1.2.2.9 Biotechnological Applications of Coconut Water

Application on the body prevents prickly heat and summer boils and sub sides the rashes caused by small pox, chicken pox and measles etc. (Andrade *et al.*, 2002).

Coconut water contains cococin which may be used in applications to support the healthy growth of human tissues as well. The product is useful in hair care formulations and in rejuvenative topical preparations to nourish condition, soothe, and moisturize the tissues. Its convenient powder form readily blends into all types of cosmetic preparations (Tulecke, 1991).

The coconut is common and a daily food in some but not all parts of the tropics. It is well adapted and can be grown almost anywhere. The tree itself is versatile in its application and may be the most useful tree of the tropics. The fruit is used at all stages in unique ways, and is a significant source of protein and a major source of fat in the diet (Franklin, 1998).

Due to the coconut water is a sterile, a little acid solution. It contains proteins, salts, carbohydrates, vitamins, growth factors (phytohormones) and traces of phospholipids (Laguna, 1996). It has been used in many areas like cryopreservation of cells and tissues. It is an efficient medium

used to preserve and dilute goat (Nunes and Salgueiro, 1999), pig (Toniolli *et al*., 1996) and sheep semen (Guerra and Nunes, 1999), as well as murine oocyte (Blume *et al*., 1997a) and embryos (Blume *et al*., 1997b). Trace and minor element contents were determined in green coconut water for application as intravenous hydration fluid, to replace the expensive parenteral nutrition solutions for humans (Petroianu *et al*., 2004) in undeveloped regions.

The coconut water can be dreid into powder, the powdered coconut water (ACP®). This powder can be reconstituted with sterile water. After that, the biochemical characteristics of ACP® are very similar to those of fresh coconut water. The powder can be easily stored and readily sent to regions where fresh coconuts are not available. The objective of this experiment was to verify the efficacy of ACP 201® as diluent to live virus of Newcastle disease vaccine in domestic pigeons (Alexander, 1997).

2.1. Materials

2.1.1 Apparatus and Equipments

The following equipments and apparatuses were used to perform the study:

Apparatus	Company (origin)
Magnetic stirrer	Gallenkamp (England)
Autoclave	Gallenkamp (U.K.)
Micro centrifuge	Eppendorf (Germany)
Inverted light microscope	Olympus (Japan)
Distillator	GFL (Germany)
Electrical balance	Mettler (Switzerland)
Electrical incubator	Gallenkamp (Germany)
Electrical incubator	Sanyo (Japan)
Electrical oven	Gallenkamp (Germany)
Spectrophotometer	Cecil (Germany)
Millipore filter unit (0.22μm)	Millipore and Whatman (England)
Micropipette	Oxford (U.S.A.)
Refrigerator	Concord
Sensitive balance	Delta Range (Switzerland)
Shaker incubator	Sanyo (Japan)
Spectrophotometer	Apel (Japan)
pH-Meter	Meter GmbH-Teledo (England)
Camera	Olympus (Japan)

2.1.2 Chemicals

Materials used in this study were classified according to the manufacturing companies:

Materials	Company (Origin)
Beef Extract	Fluka (Switzerland)
Calcium carbonate	BDH(England)
$MgSO_4.7H_2O$	BDH (England)
$MnSO_4.4H_2O$	BDH (England)
Triammonium citrate	Merek (Germany)
Sodium acetate hydrate	Merek (Germany)
K_2HPO_4	BDH (England)
Tween-80	Sigma (U.S.A.)
Glucose	BDH (England)
Yeast extract	Biolife (Italy)
Peptone	BDH (England)
KNO_3	BDH (England)
Soluble starch	BDH (England)
NaCl	Fluka (Switzerland)
$FeSO_4.7H_2O$	BDH (England)
Agar –Agar	Difco (England)

2.1.3 Culture Media

2.1.3.1 Ready to Use Media (Manufacture Media)

Media	Company (Origin)
Brain Heart Infusion Agar	Oxoid (England)
Nutrient Agar	Biolife (Italy)
Nutrient Broth	BDH (England)
MacConkey Agar	Oxoid (England)
Muller-Hinton Agar	Himedia (India)
Brain Heart Infusion Broth	Oxoid (England)
Salmonella –Shigella Agar (SS agar)	Oxoid (England)
Agar –Agar	Difco (England)
MacConky Broth	Oxoid (England)

2.1.3.2 Laboratory-Prepared Media

The following media were freshly prepared in the laboratory (Atlas *et al.*, 1995):

- Man-Rogoza-Sharp (MRS) Agar.
- Gauza Agar Medium.

2.1.4 Antibiotics disks

The following antibiotics discs were used in this study (NCCL, 2002):

Antibiotics	Abbreviations	Concentration (µg/disc)	Company(origin)
Chloramphenicol	C	30	Oxoid (England)
Streptomycin	S	10	Oxoid (England)
Gentamycin	CN	10	Bioanalysis (UK)
Trimethoprim	Tp	1.25	Oxoid (England)
Erythromycin	E	15	Bioanalysis (UK)
Penicillin G	P	10	Bioanalysis (UK)
Lincomycin	MY	10	Bioanalysis (UK)
Ciprofloxacin	CIP	10	Oxoid (England)

2.1.5 Solutions and Reagents

2.1.5.1 Laboratory prepared solutions and reagents:

- Normal Saline Solution: It was prepared by dissolving 0.85g of NaCl in 100ml of distilled water. The pH was adjusted to 7.0 and then sterilized by autoclaving as in (2.2.2.1) (Atlas *et al.*, 1995).

2.1.6 Bacterial Isolates

The following bacterial isolates were obtained from the Al-Nahrain University, Biotechnology department.

Bacterial Isolates	Type
E. coli	Pathogenic
Lactobacillus casei	Non Pathogenic
Salmonella typhimuirum	Pathogenic
Streptomyces spp.	Non Pathogenic
Bacillus spp.	Non Pathogenic
Pseudomonas aeruginosa	Pathogenic

2.1.7 Animals

Thirty two healthy white laboratory female mice were obtained from the National Center for Drug Control and Research, Baghdad. Animal ages ranged between 6 and 8 weeks, their weights were about 20-28gm. Animals were placed in small plastic cages, every cage size was 29 × 12.5 × 11.5 cm, and each cage contains 4 female. Floors of the cages were covered with the soft crushed wood shaving.

Basal diet was locally made of the following materials (Peter and Pearson, 1971)**:**

Product	(%) Percentage
Milled barley	24.50
Milled whert	30.00
Milled yellow corn	22.50
Milled Soya bean	15.20
NaCl	0.45
Calce stone	0.20
Animal protein	7.15

The cages were washed once a week with soap and tap water and then sterilized with 70% ethyl alcohol throughout the period of the study as it was mentioned by (Peter and Pearson, 1971) .Animals were kept under suitable environmental conditions such as room temperature (20-25ºC). The animals were fed with a suitable quantity of water and basal diet (standardpellets) according to (Vodopich and Moor, 1992).

2.1.8 Plant Material

Coconut water was obtained from coconuts (*Cocos nucifera*) that were purchased from local market.

2.1.9 Biochemical Kits used in the study

Kit	Company (origin)
Glutamic Oxaloacetic Transaminase (GOT)	Biomeriex (France)
Glutamic Pyruvic Transaminase (GPT)	Biomeriex (France)
Alkaline Phosphates	Biomeriex (France)
Triglyceride	Biomeriex (France)
High Density Lipoprotein- Cholesterol (HDL-C)	Biomeriex (France)

2.1.10 Plasmid

The plasmid pEGFP (Living Colors Family of Vectors, B-D Biosciences) from (Bio-RAD^{TM}) company was used in this study.

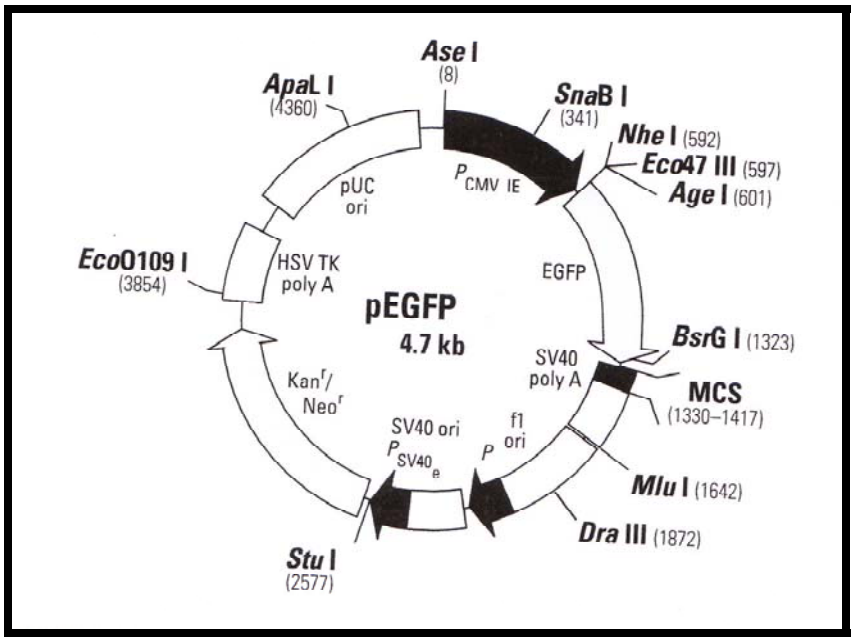


Figure (2-1): Plasmid pEGFP (Chalfie, 1994).

Restriction Map and Multiple Cloning Site (MCS) of pEGFP. All restriction sites shown are unique. The Xba I and Bcl I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with these enzymes, you will need to transform the vector into a dam– host and make fresh DNA.

Description

pEGFP encodes a red-shifted variant of wild-type GFP (Prasher, 1992 ; Chalfie,1994 ;Inouye & Tsuji,1994) .Which has been optimized for brighter fluorescence and higher expression in mammalian cells. pEGFP encodes the GFPmut1 variant (Cormack, 1996) .

Which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more

than 190 silent base changes which correspond to human codon-usage preferences (Haas, 1996).

Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site to further increase the translation efficiency in eukaryotic cells (Kozak, 1987).

The MCS in pEGFP is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neor), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in E. coli. The pEGFP backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single stranded DNA production.

Location of features (Prasher, 1992).

• Human cytomegalovirus (CMV) immediate early promoter: 1–589 Enhancer region: 59–465; TATA box: 554–560 Transcription start point: 583 C→G mutation to remove Sac I site: 569

• Enhanced green fluorescent protein gene Kozak consensus translation initiation site: 606–616 Start codon (ATG): 613–615; Stop codon: 1408–

1410 Insertion of Val at position 2: 616–618 GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810 His-231 to Leu mutation (A→T): 1307 Last amino acid in wild-type GFP: 1327–1329.

• MCS: 1330–1417.

• SV40 early mRNA polyadenylation signal Polyadenylation signals: 1550– 1555 & 1579–1584; mRNA 3' ends: 1588 & 1600.

• F1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of EGFP).

• Bacterial promoter for expression of Kanr gene –35 region: 2164–2169; –10 region: 2187–2192 Transcription start point: 2199.

• SV40 origin of replication: 2443–2578.

• SV40 early promoter. Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419.21-bp repeats: 2423–2443, 2444–2464, & 2466–2486

Early promoter element: 2499–2505

Major transcription start points: 2495, 2533, 2539 & 2544

• Kanamycin/neomycin resistance gene

Neomycin phosphotransferase coding sequences:

Start codon (ATG): 2627–2629; stop codon: 3419–3421

G→A mutation to remove Pst I site: 2809

C→A (Arg to Ser) mutation to remove BssH II site: 3155

• Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal. Polyadenylation signals: 3657–3662 & 3670–3675

• pUC plasmid replication origin: 4006–4649

Primer Locations (Kozak, 1987).

• EGFP-N Sequencing Primer (#6479-1): 679–658

• EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in E. coli (Gorman, 1985).

• Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.

• Selectable marker: plasmid confers resistance to kanamycin (30 μg/ml) to E. coli hosts.

• E. coli replication origin: pUC

• Copy number: ≈500

• Plasmid incompatibility group: pMB1/ColE1

2.2 Methods

2.2.1 Sterilization Methods (Baily *et al.*, 1990)

Three methods of sterilization were used:

2.2.1.1 Autoclaving

Media and solutions were sterilized by autoclaving at 121°C (15Ib/inch²) for 15 minutes unless otherwise mentioned.

2.2.1.2 Dry heat sterilization (Oven)

Electric oven was used to sterilize glassware and other instruments at 160-180°C for 2-3 hrs.

2.2.1.3 Membrane Filter Sterilization (Filtration)

Millipore filters (0.22μm) were used to sterilize coconut water.

2.2.2 Media Preparation

2.2.2.1 Ready to make media

Media listed in (2.1.3.1) were prepared according to the instructions of the manufacturer:

A. Brain Heart infusion broth (BHI):

It was prepared by dissolving 37 g of BHI in 950 ml of distilled water, then pH was adjusted to 7.0, the volume was completed to 1 litter, and sterilized by autoclaving.

B. MacConkey Agar:

It was prepared by dissolving 50g of MacConkey Agar in 950ml of distilled water, then pH was adjusted to 7.0, the volume was complete to 1 litter, and sterilized by autoclaving.

C. Muller Hinton Agar:

It was prepared by dissolving 35 g of Muller Hinton Agar powder in 950 ml of distilled water, then pH was adjusted to 7.0 the volume was completed to 1 litter, and sterilized by autoclaving.

D. Nutrient Agar:

It was prepared by dissolving 28g of Nutrient Agar powder in 950 ml of distilled water, then pH was adjusted to 7.0, the volume was completed to 1 litter, and sterilized by autoclaving.

E. Nutrient broth:

It was prepared by dissolving 8g of Nutrient broth powder in 950 ml of distilled water, then pH was adjusted to 7.0, the volume was completed to 1 litter, and sterilized by autoclaving.

F. *Salmonella –Shigella* Agar (S-S agar)

It was prepared by dissolving 60g of (S-S agar) powder in 950 ml of distilled water, then the pH was adjusted to 7.0, volume was completed to 1 litter, and sterilized by autoclaving.

2.2.2.2 Laboratory prepared media

2.2.2.2.1 MRS Agar (Deman *et al.*, 1960)

This medium was prepared to be consisted of the following components:

Ingredient	Weight (g)
Peptone	10
Beef extract	10
Yeast extract	5
Glucose	20
K2HPO4	2
Sodium acetate hydrate	5
Triammonium citrate	0.2
MgSO4.7H2O	2
MnSO4.4H2O	0.05
Agar	1.5

All ingredients were dissolved in 950ml of distilled water, then 1ml of Tween-80 was added, pH was adjusted to 7.0, the volume was then completed to 1000ml and sterilized by autoclaving.

2.2.2.2.2 Gauza Agar Medium (Komagata, 1989)

This medium was prepared to be consisted of the following components:

Ingredient	Weight (g)
KNO_3	1
Soluble Starch	20
$MgSO_4.7H_2O$	0.5
$FeSO_4.7H_2O$	0.01
NaCl	0.5
K_2HPO_4	0.5
Agar	15

All ingredients were dissolved in 950ml distilled water then the volume was completed to 1000ml with distilled water and sterilized by autoclaving**.**

2.2.2 Maintenance of bacterial isolates (Conteras *et al.*, 1997; Maniatis *et al.*, 1982; Johnson *et al.*, 1988)

A. Short term storage:

Bacterial isolates were maintained for period of few weeks on the following agar plates, wrapped tightly with parafilm and stored at 4˚C.

Isolates	Medium
E. coli	MacConky agar
Lactobacillus casei	MRS agar
Salmonella typhimurium	S-S agar
Psedomonus aeruginosa	Nutrient agar
Streptomyces spp.	Gauza agar
Bacillus spp.	Nutrient agar

B. Medium term storage:

Bacterial isolates were maintained as stab culture for months; such cultures were prepared in small screw capped bottles containing (5-7) ml of nutrient agar as slant and stored at 4˚C.

C. Long term storage:

Bacterial isolates were stored for many years in medium containing 20% glycerol at low temperature without loose in viability; this was done by adding 20 ml of glycerol to 80 ml of nutrient broth in small screw-capped bottles and stored at -20 ˚C.

2.2.4. Genetic Study

2.2.4.1 Recombinant DNA Vector Specifications and Method of Application

The plasmid pEGFP (Living Colors Family of Vectors, B-D Biosciences-Clontech) having a mass of 0.2µg, was selected for the testing of the capability of coconut water to facilitate expression of fluorescent genes in pEGFP plasmid.

The plasmid concentrations used were(0.1µg/ml, 0.08µg/ml, 0.06µg/ml, 0.04µg/ml, and 0.02µg/ml).

Stock Solutions

1. Plasmid stock solution was prepared by dissolving 0.2µg of lypholized pEGFP in 5ml of coconut water previously filtered through 0.22µm filter unit.

2. The following ratios were prepared from stock solution to coconut water (25:75, 20:80, 15:85, 10:90, 5:95). Each portion of stock solution was added to correspounding volume of coconut water aglass slide. Then a drop was taken from the eppendorf placed on aglass slide.
3. After place the cover clip on the slide it was examined under 100x magnifaction of an inverted microscope supplied with dichroic filter set for measuring the fluorescent light level of pEGFP. A positive result was detected by the formation of dark green colour resembling existence of the expressed genes.

2.2.5 *In vivo* Study of Coconut Water (Biochemical Study)

2.2.5.1 Experimental Design:

In order to detect the effect of the coconut water on the metabolism of mice, thirty two mice were used in this study and were divided to subgroups, each group containing four animals to the following treatment, as it was mentioned in Fig. (2-1):

Control Group

This group was kept only on the basal diet and was injected with normal saline (0.2ml).

A- First Step

This step involves twelve female mice; every four mice were injected with different concentrations of coconut water at (0.2 ml) and kept on

basal diet for 30 days, and then they were sacrificed by heart puncture and as follows:

1. Group One: involves four female mice were injected with whole coconut water in a concentration of 100% intrapretonially.

2. Group Two: involves four female mice were injected with coconut water in a concentration of 75% intrapretonially.

Control Group: mice females were injected with distilled water intrapretonially.

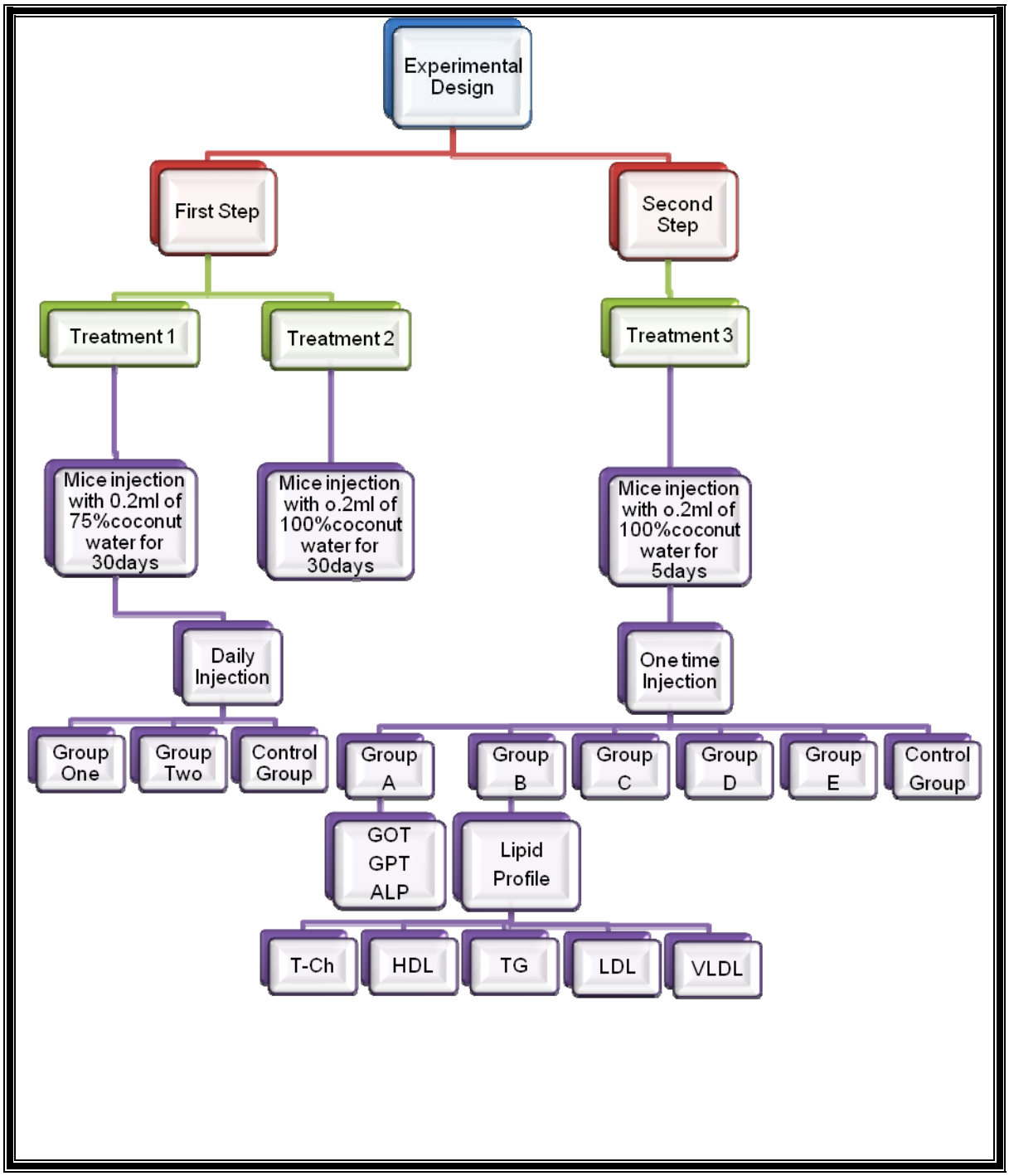
B- Second Step

This step involves twenty female mice; every four mice were injected with pure coconut water for 5days. All mice were injected (0.2ml) at the same time, and then they were sacrificed by heart puncture and as follows:

1. **Group (A):** This group was killed after 24hrs.
2. **Group (B):** This group was killed after 48hrs.
3. **Group (C):** This group was killed after 72hrs.
4. **Group (D):** This group was killed after 96hrs.
5. **Group (E):** This group was killed after 120hrs.

General Procedure

Blood samples were collected from the mice by heart puncture, serum was separated by centrifuging at 5000rpm for 10min., and then serum was stored at -20˚C until use (Silva *et al*., 1999).



Scheme (2-2): Grouping of mice and injection under study.

2.2.6 Biochemical Measurements

2.2.6.1 Enzymatic Assay

A. Assay of Liver Glutamate Oxaloacetate Transaminase (GOT) and Glutamate pyruvate Transaminase (GPT) (Reitman and Frankel, 1957)**:**

According to Reitman and Frankel (1957), blood samples were collected from the mice by heart puncture, serum was separated by centrifugation at 5000rpm for 10min., and then serum was treated as follows:

Two test tubes were used for each sample, the first one contained the blank reagent and second one contains the sample. These samples were treated as in the following:

	GPT	**GOT**
Reagent 1 Reagent 2	1ml —	— 1ml
Incubate for 5 min at 37°C.		
Serum	0.2ml	0.2ml
Mix and incubate at 37°C	— 30min	— 1hr
Reagent 3	1ml	1ml
Mix. Let stand for 20 min at room temp		
0.4N NaOH	10ml	10ml
Mixed and wait for 5min. Measure at wave length 510nm.		

Wavelength: 505nm (490 – 520)

Activities of these two enzymes in serum were estimated from the activity table attached with kit of each enzyme.

B. ALP (alkaline phosphatase)

Samples used in this test were the same of that used for GPT and GOT tests. To estimate the activity of the ALP, procedure of (kind and king, 1945) was used and as follows:

Four test tubes for each sample were prepared, the 1st contains the sample, the 2nd is the blank sample, the 3rd contain the standard sample and the 4th is the blank reagent, as shown below:

	Serum Sample	**Serum Blank**	**Standard**	**Reagent blank**
Reagent	2ml	2ml	2ml	2ml
Incubate for 5min at 37˚C.				
Serum Reagent 2	50µl —	— —	— 50µl	— —
Incubate for exactly 15min at 37˚C.				
Reagent 3	0.5ml	0.5ml	0.5ml	0.5ml
Mixed well or preferably vortex				
Reagent 4 Serum Distilled water	0.5ml — —	0.5ml 50µl —	0.5ml — —	0.5ml — 50µl
Mix. Let stands for 10min in the dark. Measure.				

Calculation= $\frac{\text{OD serum sample - OD serum blank}}{\text{OD standard}} \times n$ (Kind and King, 1945).

2.2.6.2 Analysis of Lipid Profile

2.2.6.2.1 Determination of Serum Total Cholesterol

Total cholesterol in the serum was measured by enzymatic method with the biomerux Kit, France.

Principle

The principle of this method was the lysis of the cholesterol esters to produce cholesterol and fatty acids, and then oxidized to produce the quinoemine:

Cholesterol ester $\xrightarrow{\textit{cholesterol}\text{ esterase}}$ cholesterol + fatty acid

Cholesterol $\xrightarrow{\textit{cholesterol}\text{ oxidase}}$ cholest-4-en-3-one + H_2O_2

Reagents

The reagent used in this test is mixture of:

1- Phosphate buffer	0.1 mol/L
2- Phenol	15 mol/L
3- Sodium cholate surfactant	3.74mmol/L
4- Amino antipyrine	0.5 mmol/L
5- peroxidase	≥ 1000 u/L
6- Cholesterol oxidase	≥ 200 u/L
7- Cholesterol esterase	≥ 125 u/L

Procedure

The procedure for this method was as follows:

	Reagent blank	**Standard**	**Sample**
Standard 200mg/dL	–	10µl	–
Sample	–	–	10µl
Working Reagent	1ml	1ml	1ml

After addition, the contents of every tube were mixed. Allowed to stand at room temperature for 10min. or incubated at 37˚C for 5min and the absorbency was measured at 500nm. The intensity of the produced color is directly proportional to total cholesterol concentration in the sample.

$$\text{Total cholesterol (mmol/L)} = \frac{\text{Abs. of sample at nm}}{\text{Abs. of standard at nm}} \times 5.17$$

2.2.6.2.2 Determination of Serum Triglycerides

Total triglycerides in the serum were measured by enzymatic with the (biomerieux kit, France)

Principle

Total triglyceride determination depends on formation of quinonemine by using a group of enzymes as follows:

Triglycerides $\xrightarrow{\textit{lipase}}$ **glycerol+ fatty acids**

Glycerol + ATP $\xrightarrow{\textit{glyverokinase}}$ **glycerol – 3 – phosphate+ ADP**

Glycerol – 3 – phosphate $\xrightarrow{\textit{glycerol – 3 – phosphate}}$ **H_2O_2 + dihydroxy- acetone phosphate**

H_2o_2+parachlorophenol+aminoantipyrine $\xrightarrow{peroxidase}$ **quinoneimine+H_2O+ HCL**

Reagents

The reagents used in this test are a mixture of:

1. Buffer pH 7.6 100mmol/L
2. P- Cholestrol 2.7 mmol/L
3. Magnesium 4mmol/L
4. 4- Aminoantipyrine 0.4mmol/L
5. Lipase ≥ 1000u/L
6. Glycerokinase ≥ 2000 u/L
7. Glycerol- 3- phosphate oxidase ≥ 2000 u/L
8. Peroxidase ≥ 2000 u/L
9. ATP 0.8 mmol/L
10. Glycerol 2.29 mmol/L

Procedure

The content of every tube were mixed gently after addition, allowed to stay at 20 – 25˚C temperature for 10min or incubated at 37˚C for 5min and optical density measured by spectrophotometrically at 505nm. The intensity of the produced color is directly proportional to total triglyceride concentration in the sample.

	Reagent blank	**Standard**	**Sample**
Standard 200mg/dL	–	10µl	–
Sample	–	–	10µl
Working Reagent	1ml	1ml	1ml

Calculation:

$$\text{Sample concentration (mmole/l)} = \frac{\text{Abs. of sample}}{\text{Abs. of standard}} \times n$$

(n= concentration of standard n= 2.29)

2.2.6.2.3 Determination of Serum High Lipoprotein-Cholesterol (HDL-C)

HDL – Cholesterol the serum were measured by enzymatic method using biomeriex kit, France.

Principle:

Chylomicrons, very low- density lipoprotein (VLDL) and low-density lipoprotein (LDL) contain in the specimen are precipitated by the addition of phosphotungstic acid in the presence of high – density lipoprotein (HDL) measured by enzymatic method (Wild *et al.*, 1992).

Reagents:

HDL- Cholestrol Precipitant	**Phosphotungstic acid**	**40g/L**
	$MgCL_2.6H_2O$	100g/L
	pH 6.2	1g/L

Procedure:

The working solution was the cholesterol enzymatic solution which was gently mixed with the contents of every tube; after the addition it was left to stay at 20-25˚C for 10min or incubated it for 5min at 37˚C and then optical density read by spectrophotometrically at 500nm.

	Reagent blank	Standard	Sample
Distilled water	50 µL	-	-
HDL–calibrator	-	50 µL	-
Supernatant	-	-	-
Working reagent	1 ml	1 ml	1 ml

Calculation

$$\text{HDL. Cho. (mmol/L)} = \frac{\text{Abs. of sample at nm}}{\text{Abs. of standard at nm}} \times 1.42$$

(1.42= the concentration of standard) (Wild *et al.*, 1992).

2.2.6.2.4 Determination of Serum Low Density Lipoprotein – Cholesterol (LDL)

In lipoprotein fractionation the widely accepted method for determining the LDL – cholesterol is the beta quantification procedure very low – density lipoprotein (VLDL) is separated by precipitation, because ultra centrifugation is unavailable in most routine laboratories and the produce is expensive.

Time consuming and technically demanding the nearly universal approach in clinical laboratories has been to estimate LDL – cholesterol from the formula of (Legro *et al.*, 1999).

After the measurement of total cholesterol, triglycerides and HDL – cholesterol, LDL – cholesterol is calculated as total cholesterol minus

very low – density lipoprotein VLDL (estimated as triglyceride ÷ 5) – with those obtained by lipoprotein fractionation. When triglyceride was <0.2g/dl 90% estimated LDL – cholesterol vale accepatable, with ± 10% measured values.

$$\frac{TG}{5} = VLDL$$

LDL = total Cholesterol – (HDL - cholesterol + VLDL) (Legro *et al.*, 1999).

2.2.7 Determining the Efficiency of Coconut Water Media (Prescott *et al.*, 1999)

1. In order to determine the growth efficiency of microorganisms (pathogenic and nonpathogenic bacteria) on coconut water as a growth medium, different ratios of coconut water to distilled water (0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 40:60, 30:70, 20:80, 10:90) were used as a solid and liquid medium for growth of 1.5×10^5 (cfu/ml) of the test bacteria (*E. coli*, *Lactobacillus casei*, *Salmonella typhimuirum*, *Streptomyces*, *Bacillus spp.* and *Psedomonus aeruginosa*).

2. Serial dilutions for the tubes containing liquid growth were prepared and optical density at (O.D 600) nm was measured for each dilution.

3. Viable count was measured for each dilution by spreading 0.1ml of the suitable dilution on nutrient agar plates, and incubated at 37ºC for 24hrs (1.5×10^5cfu/ml).

2.2.8 Antibiotic sensitivity test of bacterial isolates (Atlas *et al*., 1995)

After determining the best concentration, coconut water medium was compared to Muller-Hinton medium to test the antibiotic activity and inhibition zones formed in each medium.

Sensitivity of test microorganisms (*E. coli*, *L. casei*, *Sal. typhimuirum*, *S. spp.*, *B. spp.*and *P. aeruginosa*) to different antibiotics, (Trimethoprim (Tp), Erythromycin (E), Penicillin G (P), Lincomycin (MY), Ciprofloxacin (CIP), Chloramphenicol (C), Gentamicin (CN) and Streptomycin (S), was examined using the modified disc diffusion method. Ten ml of coconut water medium concentration was inoculated with bacterial isolate, and incubated at 37ºC for 18hrs, 0.1ml of [(1×10^5, 1×10^6 CFU/ ml) freshly broth (Growth of bacteria was monitored by McFarland tube No. 5 turbidity standard, which as equivalent to bacterial concentration for inoculums (1×10^5, 1×10^6 CFU/ml)] transferred to Muller–Hinton agar plate and streaked by sterile cotton swab three times by rotating the plate approximately 60mm between streaking to ensure even distribution of the inoculums, the inoculated plates were placed at room temperature for 10min to allow absorption of excess moisture, then antibiotic disks were applied by sterile forceps on the surface of plates and incubated at 37ºC for 18hrs in an inverted position.

After incubation, the diameter of inhibition zone was measured (clear area around disks) by ruler which indicates the sensitivity of bacteria to that antibiotic and results were compared with (NCCLs, 2002).

2.2.9 Statistical analyses

Statistical analysis was performed to compare two different groups by using paired student test. Statistical significance was determined at $P<0.05$ (Almohammed *et al*., 1986).

3.1 Genetic Study

3.1.1 Recombinant DNA Vector Specifications and Method of Application

Coconuts may be the best choice for the production of recombinant proteins (Ma *et al*., 2003). This method showed with simple inverted pEGFP microscopy, that coconut water expressed foreign DNA sequences. Since hundreds of milliliters could be extracted from each coconut and could be exploited for large-scale, industrial production of recombinant proteins using bioreactor technology. Furthermore, as coconut water nuclei are naked, devoid of cell membranes, the micropropagation of genetically engineered plant clones is greatly facilitated (Taylor and Fauquet, 2002). Twenty coconuts of (87±12) mm diameter, (99±18) mm length and (130±37) ml (mean ±S.D.) were used. Fig.(3-1) Illustrates typical syncytial nuclei (using inverted microscopic). Syncytial endosperm contains cell-free, naked nuclei that continuously divide because one of the functions of the nuclei is to form new layers of endosperm cells (Cutter and Freeman, 1952).

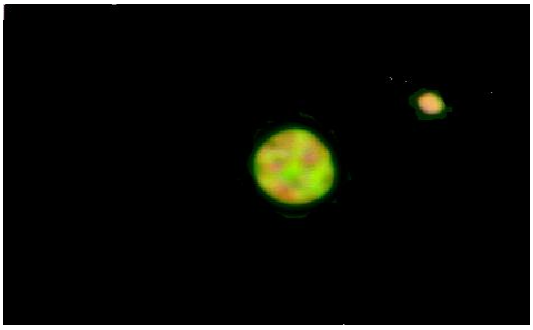
Results showed that 0.1µg/ml gave dark green color after 4hrs and 5hrs. Fig. (3-1 B and C) showed an inverted microscopy image taken after 4 and 5hrs respectively of pEGFP application.

The entry of pEGFP into the naked nucleus, exclusively via the nuclear pores, is assisted by the nuclear localization signals present in the transcription factors attached to the plasmid (Bustamante, 2002).

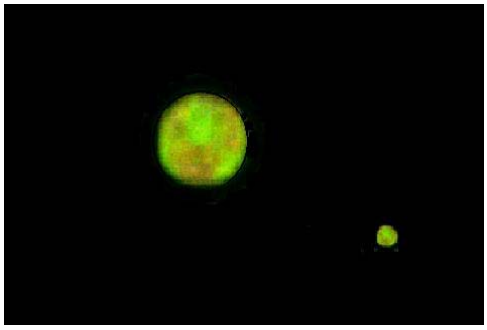
The large volume of water in each coconut translates into significant savings because no culture broth is needed (as is the case for microbial

fermentation systems) (Cantoni *et al.*, 1993; Tao *et al.*, 1997; Chaturvedi *et al.*, 2003).

A



B



C

Figure (3-1): Coconut water contains active nuclei devoid of cellular membranes.

(A) Conventional light microscope (100X) images of cell-free, naked nuclei.

(B and C) Inverted microscopy (100X) supplied with fluorescent filter showing pEGFP expression after (4, 5) hrs respectively application of 0.1µg/ml pEGFP.

3.2 Aims of Biochemical Measurements

3.2.1 Differences between GOT and GPT which is sensitive to liver diseases

3.2.1.1 Step 1 (30days)

3.2.1.1.1 Glutamic Oxaloacetic Transaminase (GOT)

This enzyme also known as aspartate aminotransaminase is widely distributed with high concentrations in the heart, liver, skeletal muscle, kidney and erythrocytes. Damage to any of these tissues cause raised levels of GOT (Tieta *et al*., 1986). Results showed that there were no significant differences in GOT activity in blood serum of adult mice females which were injected with coconut water at the concentrations of 100 and 75% respectively. GOT activity reached (199.5±7.99)U/l at the concentration 100% when compared with its level in blood samples of control group (193.02±3.26U/l), while GOT activity reached (198.6±6.01)U/l at the concentration of 75% when compared with its level in blood samples of the control group. This revealed that coconut water has no effect on liver functions. This result agreed with Bergmeyer, (1974), who revealed that serum GOT levels in healthy subjects were low, but the levels were significantly elevated in a number of clinical conditions such as acute and chronic hepatitis, obstructive jaundice, carcinoma of the liver and myocardial infraction.

3.2.1.1.2 Glutamic Pyruvic Transaminase (GPT)

This enzyme also known as alanine aminotransaminase is found in higher concentration in liver and to a lesser extent in skeletal muscle, kidney and heart (Wong *et al*., 2000). Results showed that there were no

significant differences in GPT activity in blood serum of adult mice females which were treated with coconut water at the concentrations of 100 and 75% respectively. GPT activity reached (67.52±4.847)U/l at the concentration 100% when compared with its level in blood samples of the control group (66.03±3.529U/l), while GPT activity reached (67.37±4.897)U/l at the concentration of 75% when compared with its level in blood samples of the control group (66.03±3.529U/l). No increase in the levels of the hepatic enzyme GPT was detected, so no liver damage occurred. These results proved that coconut water had no effect on the hepatic cells. These results agreed with Pratt and Kaplan, (2001) who revealed that serum GPT values more than the normal limit always indicate an acute hepatocellular damage of viral, toxic or circulatory origin, in most types of liver diseases, GPT activity is higher than that of GOT, and it is more sensitive and specific in the detection of liver diseases.

3.2.1.1.3 Alkaline Phosphatase (ALP)

Results showed that there were no significant differences in ALP activity in blood serum of adult mice females which were injected with coconut water at the concentrations of 100 and 75% respectively. ALP activity reached (64.037±3.802)U/L at the concentration 100% when compared with its level in blood samples of control group (60.658±5.581U/L), while ALP activity reached 60.658±5.581U/l at the concentration of 75% when compared with its level in blood samples of the control group (61.517±3.926U/L). These results proved that coconut water has no effect to prevent the hepatic cells and no changes in the

intestinal activity of it. These results agreed with Mathur and Dive (1981), as shown in fig.(3-2).

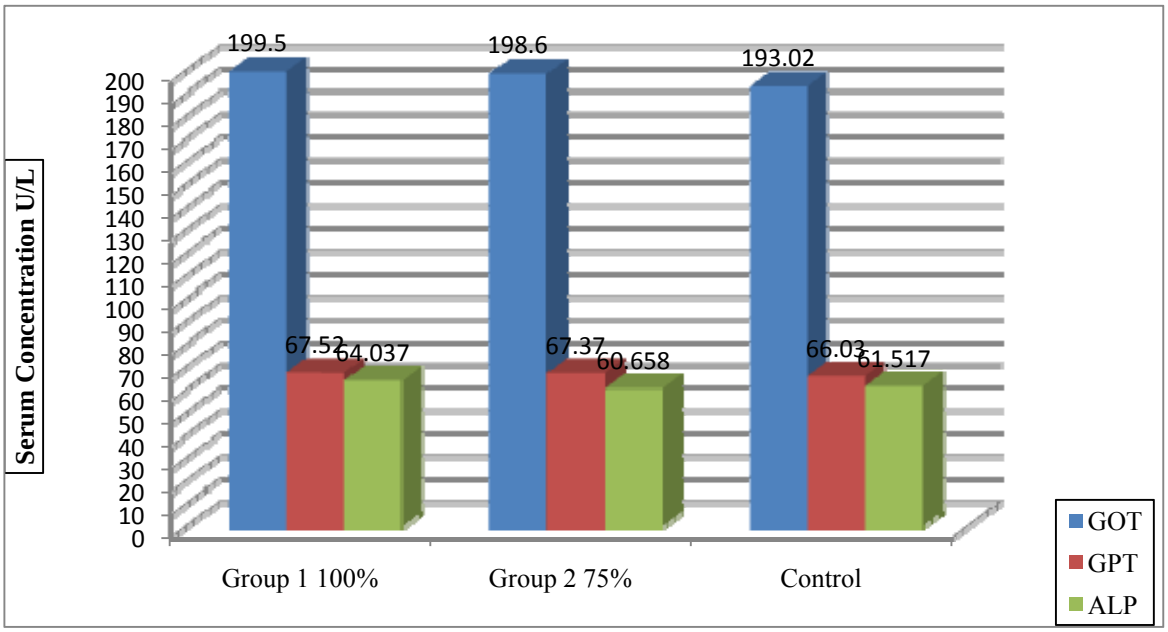


Figure (3-2): Serum GOT, GPT and ALP levels in mice injected intrapretonially with two concentrations of coconut water (100 and 75)% and control group.

3.2.1.1.4 Lipid profile

Serum total cholesterol and other lipid profile constituents in the control and in the blood serum of mice females groups were shown in fig. (3-3). When lipid profile contents were determined the results showed no significant differences in blood serum of adult mice females, this may be indicated that the coconut water consumption reduced plaque formation in arteries and thus led to reduce the risk of heart attack and stroke, also reduced high blood pressure and increased circulation (Alleyne, 2005).

Coconut water contains form of dietary fiber and amino acids that help moderate sugar absorption and improve insulin sensitivity (Santoso *et al.*, 1996).

A- **Serum Total Cholesterol**

Results showed that there were no significant differences in serum total cholesterol concentration of about blood serum of adult mice females which were injected with coconut water at the concentrations of 100 and 75% respectively. Serum total cholesterol activity reached (134.31±9.13)mg/dL at the concentration of 100% when compared with its level in blood samples of control group (132.04±6.04mg/dL), serum total cholesterol activity reached (136.27±5.34)mg/dL at the concentration of 75% when compared with its level in blood samples of the control group (132.±6.04mg/dL).

B- Serum Triglyceride (TG)

Results showed that there were no significant differences in serum TG concentration of blood serum of adult mice females which were injected with coconut water at the concentrations of 100 and 75% respectively. Serum TG activity reached (110.5±8.22)mg/dL at the concentration of 100% when compared with its level in blood samples of the control group (111.39±3.31mg/dL), serum TG activity reached 115.11±3.89mg/dL at the concentration 75% when compared with the control group (111.39±3.31mg/dL).

C- Serum HDL – cholesterol

On the other hand, results showed that there were no significant differences in serum HDL – cholesterol of blood serum in mice females, which reached (25.452 ± 3.212) mg/dL at 100% concentration of it when compared with its level in blood samples of the control group (22.203 ± 2.528) mg/dL, also the serum HDL – cholesterol reached (25.25± 3.656)mg/dL at 75% concentration of it when compared with its level in blood samples of the control group (22.203 ± 2.528 mg/dL).

D- Serum LDL and VLDL - Cholesterol

The results observed which associated with lipid profile led to no significant differences in level of LDL cholesterol in blood serum of mice females, LDL level reached (138.5 ± 10.698) mg/dL at 100% concentration when compared with its level in blood samples of the control group (131.965 ± 7.906 mg/dL), also the serum LDL reached (131.965 ± 7.906)mg/dL at 75% concentration of it when compared with its level in blood samples of the control group (131.965 ± 7.906 mg/dL). Results revealed that VLDL values referred no significant differences, which reached (22.1±1.644)mg/dL at 100% concentration of coconut water when compared with its level in blood samples of the control group (22.278±0.662mg/dL), and LDL level reached 23.02±0.778mg/dL at 75% concentration of coconut water when compared with its level in blood samples of the control group (22.278±0.662mg/dL).

- The VLDL was measured in serum of mice females and the control group according to the following equation.

$$\frac{TG}{5} = VLDL$$

LDL – cholesterol = total cholesterol – (HDL + cholesterol + VLDL)

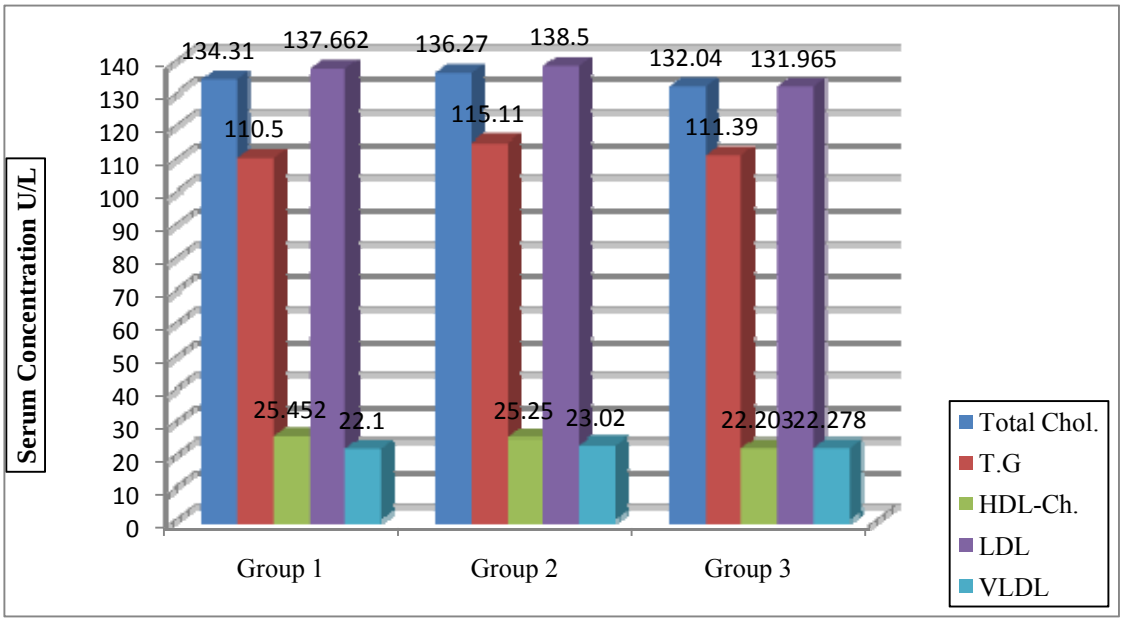


Figure (3-3): Serum lipid profile (Total cholesterol, TG, HDL- cholesterol LDL and VLDL) in female mice injected intrapretonially with coconut water at the concentrations of coconut water (100 and 75%) and control group.

3.2.1.2 Step 2 (5days)

3.2.1.2.1 Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT)

Group A was injected with 100% concentration of coconut water after 24hrs, results showed that injection of mice with coconut water led to no significant differences in the levels of GOT and GPT in blood serum of mice which reached (199.03±5.58)U/L, (74.820±3.085)U/L respectively, when they were compared with their levels in blood samples of the control group (193.02± U/L, 66.03±3.529U/L).

While group was injected with 100% concentration of coconut water after 48hrs, results showed that injection of mice with coconut water led to no significant differences in the levels of GOT and GPT in blood

serum of female mice of reached (203.37±11.01U/L, 76.200±5.504U/L) respectively, when they were compared with their levels of blood samples of the control group (193.02± U/L, 66.03±3.529U/L).Group C was injected with 100% concentration of coconut water, results were calculated after 72hrs and indicated that injection of mice with coconut water led to no significant differences in the levels of GOT and GPT in blood serum of mice which reached (196.35±2.77)U/L,(69.553±4.355)U/L respectively, when compared with their levels blood samples of the control group (193.02± U/L, 66.03±3.529)U/L, while group D was injected with 100% concentration of coconut water, results were calculated after 96hrs indicated that injection of mice with coconut water which led to no significant differences in the levels of GOT and GPT in blood serum of female mice reached (192.63±3.24)U/L, (70.815±4.077)U/L respectively, when compared with their levels in blood samples of the control group (193.02± U/L, 66.03±3.529U/L).

Group E was injected with 100% concentration of coconut water after 120hrs, results were determined and indicated that injection of mice with coconut water led to no significant differences in the levels of GOT and GPT in blood serum of female mice reached (191.3±5.31)U/L, 70.553±1.746U/L respectively, when there were compared with its level blood samples of the control group (193.02± U/L, 66.03±3.529U/L) as represented in fig. (3-4).

3.2.1.2.2 Alkaline Phosphatase (ALP):

In order to know the effect of coconut water on the ALP level in blood serum in female mice, group A mice were injected with coconut water at the concentration 100%, results showed that there were no significant

differences ($p \leq 0.05$) in ALP activity in blood serum of adult mice females, after 24hrs of injection ALP activity reached (63.622±4.884U/L) when compared with its level in blood samples of the control group (61.517±3.926U/L). The effect of coconut water on the ALP level in blood serum of female mice in group B was determined when the mice were injected with coconut water of 100% concentration of which results showed that there were no significant differences in ALP activity in blood serum of adult female mice when they were killed after 48hrs. ALP activity reached (62.005±4.595U/L) when compared with its level in blood samples of the control group (61.517±3.926U/L).

The results represented in fig. (3-4) effect of coconut water on the ALP level in blood serum of female mice was calculated when the mice of group C were injected with 100% concentration of coconut water, mice were killed after 72hrs and results revealed that there is a significant decrease ($p \leq 0.05$) in level of serum ALP (56.007 ±U/L) in comparison with its level in serum of control group (61.517±3.926 U/L).

The effect coconut water on the level of ALP in blood serum of female mice is determined after mice were injected with 100% concentration of coconut water, the results shown in fig. (3-4) of group D were calculated after 72hrs revealed that there is a significant decrease ($p \leq 0.05$) in level of serum ALP (56.538 ±7.193U/L) in comparison with its level in serum of control group (61.517±3.926U/L).

Results from fig. (3-4) showed the effect coconut water on the ALP level in blood serum of female mice in group E when injected with 100% concentration of coconut water, results indicated that after 72hrs there was a significant decrease ($p \leq 0.05$) in level of serum ALP (56.007

±U/L) in comparison with its level in serum of control group (49.88±3.17U/L).

These results showed that coconut water had a slight effect on ALP level and the changes in ALP levels had no effect on the body.

These results represented that coconut water had no effect on the hepatic cells and did not change the intestinal activity. These results agreed with Mathur and Dive (1981).

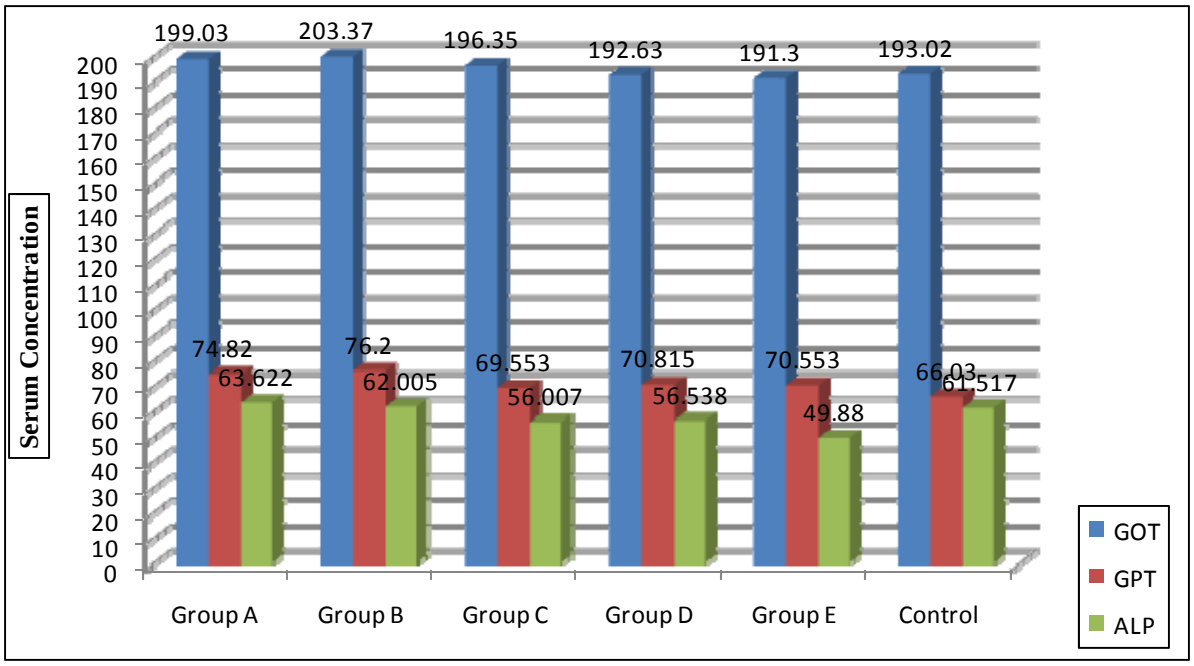


Figure (3-4): Serum GOT, GPT and ALP levels in female mice injected intrapretonially with coconut water at the concentration 100% after (24, 48, 72, 96 and 120hrs) and control group.

3.2.1.1.4 Lipid profile

A- Serum Total Cholesterol

Results in fig. (3-5) showed no significant differences in serum total cholesterol concentration in blood serum of adult female mice which

were injected with coconut water at the concentration 100%. Serum total cholesterol activity reached (130.02±2.96)mg/dL after 24hrs, and reached (129.78±6.49)mg/dL after 48hrs, while serum total cholesterol levels decreased significantly ($p \leq 0.05$) after 72, 96 and 120hrs respectively reaching (121.6±3.52)mg/dL, (119.99±3.21)mg/dL, and (119.24±2.8)mg/dL after 120hrs. When compared with its level in blood samples of the control group (132.04±6.04mg/dL), these significant differences revealed that the decrease in serum cholesterol level did not have bad effect on the heart and blood pressure (Alleyne, 2005), also results showed that coconut water contain forms of dietary fiber and amino acids that help moderate sugar absorption and improve insulin sensitivity (Sanoto *et al.*,1996).

B- Serum Triglyceride (TG)

Results showed that there were no significant differences ($p \leq 0.05$) in TG concentration in blood serum of adult female mice which were injected with coconut water at the concentration 100%. TG activity reached (103.92±8.00)mg/dL after 24hrs, and significant differences ($p \leq 0.05$) decreased reaching (99.00±6.72)mg/dL after 48hrs, while serum TG levels decreased significantly ($p \leq 0.05$) after 72, 96 and 120hrs respectively reaching (93.61±3.17)mg/dL, (90.38±2.49)mg/dL, and (88.36±7.29)mg/dL. When compared with its level in blood samples of the control group (132.04±6.04mg/dL) (Fig. 3-5), these significant differences revealed that the decrease in serum cholesterol level did not have bad effect on the heart and blood pressure (Alleyne, 2005).

C- Serum HDL – cholesterol

On the other hand, results in Fig. (3-5) showed there were no significant differences in serum HDL – cholesterol of blood serum in mice females, which reached (20.897±1.836)mg/dL at the concentration 100% of coconut water after 24hrs, while after 48hrs differences reached (22.120±3.082)mg/dL. While serum HDL cholesterol levels decreased significantly ($p \leq 0.05$) after 72, 96 and 120hrs respectively reaching (20.113±1.293)mg/dL, (20.458±2.027)mg/dL and (20.757±3.099)mg/dL when compared with its level in blood samples of the control group (22.203±2.528 mg/dL).

D- Serum LDL and VLDL - Cholesterol

Results showed no significant differences in the level of LDL cholesterol in blood serum of mice females, results showed that there were significant differences ($p \leq 0.05$) reached (130.137±3.196)mg/dL when coconut water was injected at the concentration 100% after 24hrs of injection. After 48hrs differences reached (113.062±8.228)mg/dL, while they reached (110.173±4.17)mg/dL after 72hrs and (103.947±4.769)mg/dL at 96hrs, while after 120hrs they reached (104.669±4.441)mg/dL when compared with its level in blood samples of the control group (131.965±7.906mg/dL). VLDL value referred no significant differences after 24hrs as shown in fig. (3-5) reaching (20.78±1.6)mg/dL when coconut water was injected at the concentration 100% while significant differences ($p \leq 0.05$) reached (19.8±1.344mg/dL) after 48hrs, differences reached (18.72±0.643mg/dL) after 72hrs and (18.07±0.498mg/dL) after 96hrs. Significant differences reached

(17.67±1.458mg/dL) after 120hrs when compared with its level in blood samples of the control group (22.278±0.662mg/dL).

❖ The VLDL was measured in serum of mice females and the control group according to the following equation.

$$\frac{TG}{5} = VLDL$$

LDL – cholesterol = total cholesterol – (HDL – cholesterol + VLDL)

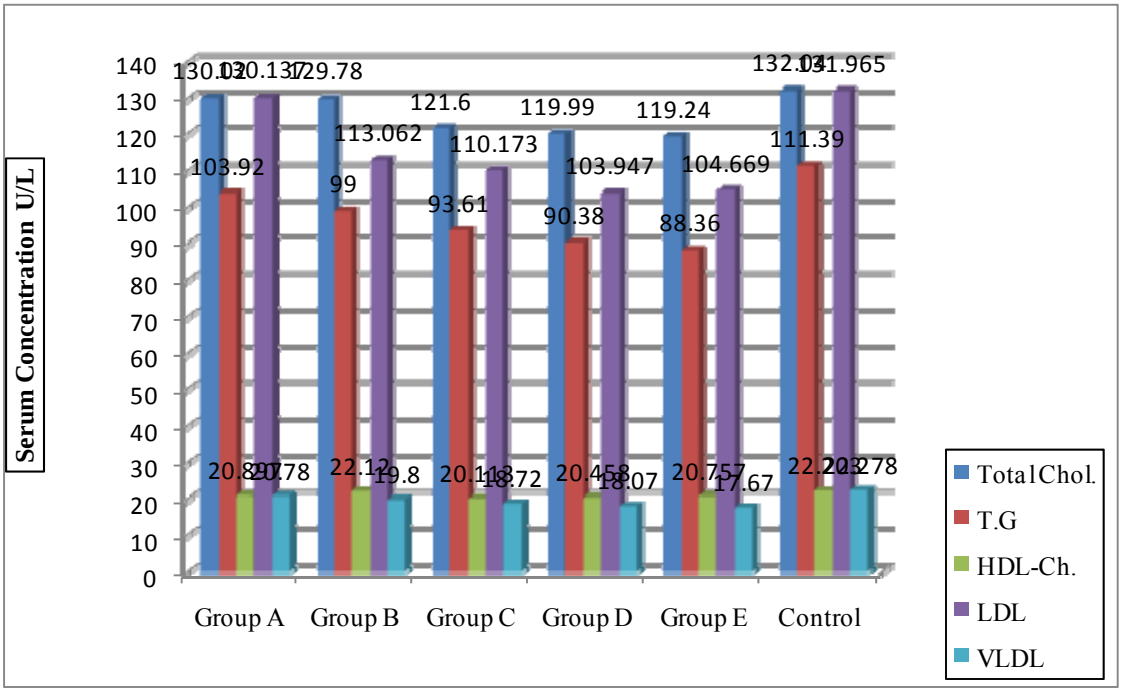


Figure (3-5): Serum lipid profile (Total cholesterol, HDL, TG, LDL and VLDL) in female mice injected intrapretonially with coconut water at concentration of 100% after (24, 48, 72, 96 and 120) hrs and control group.

3.3 Determining the Efficiency of Coconut Water Media

In order to determine the growth efficiency of (pathogenic and nonpathogenic bacteria) on coconut water as a growth medium, different ratios of coconut water to distilled water (100:0, 90:10, 80:20, 70:30, 60:40, 50:50, 40:60, 30:70, 20:80, 10:90) were prepared as a solid and liquid medium for growth of the tested bacteria (*E. coli*, *Lactobacillus casei*, *Salmonella typhimuirum*, *Streptomyces spp.*, *Bacillus spp.* and *Pseudomonas aeruginosa*).

When grown on the selective culture media (Nutrient Agar, Brain Heart Infusion Agar, MacConkey Agar, Gauza Agar and S-S Agar), the tested bacterial isolates (*E. coli*, *Lactobacillus casei*, *Salmonella typhimuirum*, *Streptomyces spp.*, *Bacillus spp.*and *Pseudomonas aeruginosa*) showed lower growth rates when compared to their growth rate on coconut water medium as shown in table (3-1).

Table (3-1): Number of colonies of tested bacteria when grown on selective media.

Media	*Sal. typhimurium*	*P. aeruginosa*	*E. coli*	*S. spp.*	*B. spp.*	*L. casei*
Nutrient Agar	43	47	45	46	50	44
Brain Heart Agar	41	48	46	42	44	43
MacConkey Agar	46	49	41	43	44	45
Gauza Agar	48	50	46	45	42	49
S-S Agar	49	43	43	48	46	49

N=3

Results showed that the highest growth rate of the bacterial isolates (*E. coli*, *Lactobacillus casei*, *Salmonella typhimuirum*, *Streptomyces spp.*, *Bacillus spp.* and *Pseudomonas aeruginosa*) on coconut water media at the concentration (100%), growth efficiency is a concentration dependent as shown in table (3-2).

Table (3-2): Number of colonies of tested bacteria on coconut water at different concentrations.

Coconut water concentration%	***Sal.typhimurium***	***P. aeruginosa***	***E. coli***	***S. spp.***	***B. spp.***	***L. casei***
100%	128	130	135	133	139	142
90%	122	123	125	126	124	135
80%	115	112	110	113	116	120
70%	107	103	101	106	102	109
60%	95	98	95	92	90	97
50%	83	80	81	85	83	84
40%	71	68	65	73	71	75
30%	63	61	58	60	63	64
20%	55	52	51	53	52	56
10%	46	48	43	44	40	45

N=3

When serial dilutions were prepared and optical density was measured, results revealed that the best dilutions were 10^{-5} and 10^{-6} (Optical Density of cell suspension for each isolate was ranged between 0.97 and 0.65), as shown in table (3-3).

The results revealed that coconut water medium can be used as preserving medium to the tested bacterial isolates for a long period of time without using any preserving substances because coconut water

contains a variety of minerals, antioxidants, aminoacids, enzymes, growth factors and other nutrients (Santoso *et al.*,, 1996).

Table (3-3): Number of colonies of tested bacteria at the concentrations (10^{-5} and 10^{-6}) on different concentrations of coconut water and on selective media.

Bac. / Media	*P.*		*Sal.*		*E.*		*B.*		*S.*		*L.*	
	10^{-5}	10^{-6}	10^{-5}	10^{-6}	10^{-5}	10^{-6}	10^{-5}	10^{-6}	10^{-5}	10^{-6}	10^{-5}	10^{-6}
c. w. 100%	**155**	**145**	**147**	**144**	**200**	**188**	**177**	**165**	**168**	**158**	**155**	**148**
c. w. 90%	**143**	**138**	**143**	**140**	**190**	**180**	**171**	**160**	**163**	**152**	**149**	**144**
c. w. 80%	**137**	**134**	**135**	**131**	**177**	**169**	**165**	**159**	**157**	**148**	**145**	**137**
c. w. 70%	**132**	**127**	**130**	**127**	**170**	**160**	**155**	**153**	**152**	**142**	**140**	**131**
c. w. 60%	**125**	**118**	**122**	**120**	**164**	**155**	**143**	**138**	**138**	**133**	**123**	**119**
c. w. 50%	**117**	**110**	**115**	**110**	**159**	**149**	**133**	**127**	**120**	**110**	**112**	**109**
c. w. 40%	**111**	**104**	**109**	**102**	**151**	**147**	**120**	**113**	**105**	**99**	**101**	**88**
c. w. 30%	**99**	**93**	**104**	**94**	**145**	**139**	**105**	**96**	**87**	**83**	**85**	**77**
c. w. 20%	**90**	**88**	**95**	**88**	**134**	**129**	**94**	**87**	**77**	**73**	**73**	**67**
c. w. 10%	**85**	**83**	**85**	**80**	**128**	**122**	**82**	**77**	**67**	**60**	**62**	**58**
N. B.	**79**	**74**	**79**	**75**	**117**	**113**	**80**	**77**	**70**	**65**	**65**	**60**
Brain Heart Broth	**80**	**75**	**77**	**74**	**90**	**85**	**79**	**75**	**73**	**67**	**70**	**63**
Mac. Broth	**88**	**80**	**85**	**79**	**100**	**94**	**80**	**75**	**72**	**66**	**69**	**63**

n=3

3.4 Antibiotic Sensitivity

The standard disc diffusion method was used to determine the sensitivity of tested bacteria (*Sal. typhimurium*, *E. coli*, *B. spp.*, *P. aeruginosa*, *L. casei* and *S. spp.*) to different antibiotics [Trimethoprim (Tp), Erythromycin (E), Penicillin G (P), Lincomycin (MY),

Ciprofloxacin (CIP), Chloramphenicol(C), Gentamicin (CN) and Streptomycin (S)].

Results showed that the isolates varied in their resistance to antibiotics (Table 3-4). Resistance to antibiotics among isolates varied according to the genus and species of isolates and to the nature of antibiotics. In general, the isolates were highly resistant to Pencillin G (P), Lincomycin (MY) and Streptomycin (S).

They were moderate in their resistance to Chloramphenicol (C), Trimetheprim (Tp) and Gentamycin (CN). They were almost sensitive to the Ciprofloxacin (CIP) and Erythromycin (E) and these results agree with Jawetz *et al*., 1998.

Results also showed the highest diameter of inhibition zone was recorded for tested bacteria (*B. spp.*) against the antibiotic Erythromycin (E), while the lowest diameter of inhibition zone was recorded for (*P. aeruginosa*) against the antibiotic Ciprofloxacin (CIP).

Table (3-4): Diameters of inhibition zones caused by antibiotic discs against tested bacteria on Muller-Hinton agar medium.

Bacteria / Antibiotic	*Sal. Typhimurium*	*P. aeruginosa*	*B. spp.*	*L. casei*	*E. cloi*	*S. spp.*
Chloramphenicol	_	_	17mm	_	_	_
Streptomycin	_	_	_	_	_	_
Penillicin G	_	_	_	_	_	_
Ciproflaxin	22mm	14mm	22mm	_	_	26mm
Gentamycin	_	_	18mm	_	_	15mm
Erythromycin	_	_	28mm	22mm	_	15mm
Lincomycin	_	_	_	_	_	_
Trimetheprim	_	_	20mm	_	_	11mm

While the results in table (3-5) showed that inhibition zones diameters caused by antibiotic discs against tested bacteria on coconut water medium are smaller than those formed on Muller-Hinton agar medium.

Table (3-5) Diameters of inhibition zones caused by antibiotic discs against tested bacteria on coconut water agar medium 100%.

Bacteria / Antibiotic	*Sal. Typhimurium*	*P. aeruginosa*	*B. spp.*	*L. casei*	*E. cloi*	*S. spp.*
Chloramphenicol	–	–	12mm	–	–	–
Streptomycin	–	–	–	–	–	–
Penillicin G	–	–	–	–	–	–
Ciproflaxin	17mm	10mm	21mm	–	–	20mm
Gentamycin	–	–	17mm	–	–	12mm
Erythromycin	–	–	20mm	27mm	–	11mm
Lincomycin	–	–	–	–	–	–
Trimetheprim	–	–	13mm	–	–	9mm

4.1 Conclusions

1. Coconut water is a good system for recombinant protein expression.
2. *In vivo* study on mice referred that coconut water can be used instead of blood plasma.
3. Coconut water media may be used as alternative media (broth and agar medium) for growth, and freshly prepared medium for propagation of different G (+)ve and G(–)ve bacteria.

4.2 Recommendations

1- Using coconut water for different applications of recombinant DNA technology.

2- Using coconut water in plant tissue culture medium for induction of callus formation.

3- Studying the possibility of using coconut water as a growth media for microorganisms.

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الخلاصـــــــــــــــــــــــــــــــــــــــة

نظرا لاهمية ماء جوز الهند في تطبيقات التقنيات الحياتية والبايولوجية المختلفة فقد تضمنت هذه الدراسة ثلاث محاور رئيسة هي التطبيقات الوراثية، الكيموحيوية والبكتريولوجية لدراسة الصفات البايولوجية التي يتميز بها ماء جوز الهند وآفاق استخداماته المستقبلية.

تضمن الجانب الوراثي دراسة مدى قابلية ماء جوز الهند كوسط ملائم لاندماج جزيئات الدنا الغريبة بعملية الارتباط (Recombination) وتعبير جيناتها في المضيف الجديد، وقد استخدم لهذا الغرض البلازميد pEGFP الذي يعد دليلا على تمام عملية الارتباط وانتقاء الخلايا الهجينة المنتجة للبروتينات المتفلورة التي يشفر لها البلازميد.

اضيف البلازميد pEGFP بتراكيز متدرجة (0.1مايكروغرام/مل، 0.08مايكروغرام/مل، 0.06مايكروغرام/مل، 0.04مايكروغرام/مل و0.02مايكروغرام/مل) الى ماء جوز الهند الطازج لملاحظة مدى قابليته على الاندماج مع الدنا لنويات ماء جوز الهند لاختبار قدرة ماء جوز الهند على تصنيع البروتينات الارتباطية (Recombinant proteins).

وقد اشارت النتائج الى ان ماء جوز الهند كانت له القدرة على التعبير عن تعاقبات الدنا الغريبة ويمكن استثمار هذه القدرة للانتاج على مستوى عالي، والانتاج الصناعي للبروتينات باستخدام تقنية المفاعل الحيوي.

التطبيقات الكيموحيوية تضمنت دراسة تاثير ماء جوز الهند على النشاط الايضي للكبد داخل جسم اناث الفئران المختبرية البيضاء التي استخدمت في هذه التجربة، حقنت الفئران تحت بريتونياً بتراكيز مختلفة من ماء جوز الهند لفترات زمنية مختلفة.

اظهرت النتائج ان ماء جوز الهند لم يكن له اي تاثير ملحوظ على مستويات الـGPT, GOT , ALP وlipid profile في اناث الفئران عند مقارنته بمجاميع السيطرة المعاملة بـnormal saline.

تضمنت الدراسة ايضا اختبار امكانية استخدام ماء جوز الهند كوسط غذائي بديل لنمو البكتريا المرضية وغير المرضية عن الاوساط التغذوية المستخدمة عادة ومقارنتها بالاوساط التغذوية التقليدية.

اظهرت النتائج ان استخدام ماء جوز الهند بتراكيز مختلفة (10-100)% كان افضل كوسط تغذوي بالمقارنة مع اي وسط تغذوي اخر (Gauza agar, Nutrient agar, MacConkey agar) بالاعتماد على طريقة عد الخلايا.

ايضا تم في هذه الدراسة اختبار حساسية البكتيريا المرضية وغير المرضية تجاه المضادات الحياتية (Trimethoprim (Tp), Erythromycin (E), Penicillin G (P), Lincomycin (MY), Ciprofloxacin (CIP), Chloramphenicol(C), Gentamicin (CN) and Streptomycin (S)**)**.

)على وسط ماء جوز الهند التغذوي بتراكيزه المختلفة(10-100)% وقورنت النتائج مع تنمية البكتيريا على وسط مولر –هنتون . واوضحت النتائج ان حساسية البكتيريا تجاه المضادات الحياتية كانت اعلى عند تنميتها على وسط مولر- هنتون.

الاهـــــــداء

الـــــــيكِ .. لروحـــــكِ .. لذكـــراكِ .. لذكـــرى ستظلُ حيـــةً في قلبي وعقلي ما حييت .. لـــروح لم تـــزل ترعاني وهي في السمـــاء ..

أمـــــــــي

الـــى مـــن لولاه ما كنتُ لأكـــون .. الـــى من علمني كلَ شئ جميـــل .. الـــى قلبِ كان سنـــدي لآخـــر اللحـــظات .. الـــى من كـــان صديـــقي ..

أبـــــــــي

الـــى ســـندي الدائـــم ..

اخوتـــي

الـــيكـــنَ .. لمحبتكـــنَ .. لمساندتكـــنَ ..

أخواتـــي

اهـــدي ثمـــرة جهـــودي

أنســـــــــــــام

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

الرَحمٰنُ ۝ عَلَمَ القُرآنَ ۝ خَلقَ الإنسٰنَ ۝
عَلَمَهُ البَيانَ ۝ الشَمسُ والقمرُ بِحُسبانٍ ۝
والنَجمُ والشَجرُ يسجدانِ ۝ والسَماءَ رَفَعها
وَوَضَعَ المِيزانَ ۝

صدقَ اللهُ العَظِيم

سورة الرحمٰن

الآية (1-7)

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الاحيائية

دراسة وراثية، كيموحيوية و بكتريولوجية على ماء جوز الهند

رسالة

مقدمة الى كلية العلوم – جامعة النهرين

وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الاحيائية

من قبل

انســـام صبحي سبع

بكالوريوس تقانة احيائية جامعة النهرين 2005

باشراف

أ.م.د. حميد مجيد الدليمي

صفر 1430 **شباط 2009**