

**BIOCHEMICAL STUDIES ON *Solanum nigrum* L.
FRUITS EXTRACTS**

By

YASSER SAED HELMY ALY

B. Sc. (Biotechnology), Fac. Agric., Cairo Univ., Egypt, 2002

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ABSTRACT

In this work, a *Solanum nigrum* ripe fruit ethanolic extract was made up, different antioxidant examination was carried out, and the result revealed that the extract have a high content of phenols compound and a great value of total antioxidant capacity, high reducing capacity. The extract also have a power to scavenge many types of free radical such as DPPH, nitric oxide and have a ferrous ion chelating activity and prevent $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ -induced deoxyribose decomposition.

Biological experiment *in vivo* on experimental CCl_4 -intoxicated male albino rats was carried out to evaluate the extract as therapy diet, extract ingestion during CCl_4 -intoxication induced significant changes, in a dose-dependant manner, in serum total protein, albumin as well as the activity of GOT, GPT and ALP and total bilirubin content.

The values of total soluble proteins, albumin, globulin and Albumin/Globulin ratio were decreased in CCl_4 -intoxicated rats at control. These decreased values were improved by the treatment with the extract. The ingestion of the studies extract into intoxicated rats produced improvements in the Albumin/Globulin ratio which increased paralleled with increasing the extract dose. These may be due to the improvements in albumin biosynthesis.

There is a high increasing in the serum activities of GOT, GPT, GOT/GPT ratio and ALP as well as increasing serum bilirubin contents in CCl_4 -intoxicated rats indicating considerable hepato-cellular injury compared to normal healthy control. The treatment with *Solanum nigrum* ethanolic extract caused a significant improvement of the functional status of liver.

The present study on kidney function revealed that CCl_4 -ingestion caused a highly significant increase in serum uric acid contents but slightly changed serum urea (insignificant) compared to normal healthy control. The treatment of CCl_4 -intoxicated rats with *Solanum nigrum* ethanolic extract improved these disturbances in serum uric acid.

The *Solanum nigrum* ethanolic extract exerted a cytotoxic effect in dose-dependant manner. There was a significant increase in percent of dead cells with increasing of the extract concentration used. It can be reported that the using of 3000 ppm of extract in MTT assay for treatment of EAC, mice Ehrlich Ascites Carcinoma cells, and AML, human adult Acute Myeloid Leukemia cell, induce about full death of AML and EAC cells.

Key words: *Solanum nigrum* L., Black nightshades, Antioxidant, Hepatoprotective, CCl_4 , Anticancer, EAC, AML.

DEDICATION

I dedicate this work to my parents, specially my mother, for all the support they lovely offered along with my life and to my professors, my colleagues for their patience help along with the period of my post graduation and to my family, my wife, my friends.

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LIST OF APPREVIATIONS

ALP	alkaline phosphatase
ALT	serum Alanine amino transferase
AST	serum Aspartate amino transferase
CAT	Catalase
DMSO	dimethylsulphoxide
DPPH	1,1-diphenyl-2-picrylhydrazyl
EDDS	S,S-ethylenediaminedissuccinic acid
EDTA	ethylenediaminetetraacetic acid
GOT	Glutamate Oxaloacetate Transaminase
GPT	Glutamate Pyruvate Transaminase
GPx	Glutathione Peroxidase
GST	Glutathione S-transferase
HCT-116	The human colorectal carcinoma cells
Hep3B	hepatocellular carcinoma cells
HepG2	a human liver cancer cell line
HMG-CoA	hepatic 3-hydroxy-3-methylglutaryl coenzyme A
HT-29	Colon Cancer Cell Line
IC ₅₀	The half maximal inhibitory concentration
iNOS	induced nitric oxide synthesis
LDL	Low Density Lipoprotein
MCF-7	Human Breast cancer cells
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
PARP	poly ADP-ribose polymerase
PBS	phosphate buffer saline
PCA	Protocatechuric acid
PKCα	Protein kinase C alpha
ROS	Reactive Oxygen Species
SOD	Superoxide dismutase
TC	Total Cholesterol
TCA	Trichloroacetic acid
TG	Triglyceride
TNF	Tumour necrosis factor
U14	cervical cancer of tumor-bearing mice
WRL-68	a human fetal liver cell line

INTRODUCTION

In recent years, there has been a global trend toward the use of natural substances present in fruits, vegetables, oilseeds, and herbs as antioxidants and functional foods (Farr, 1997; Wang *et al.*, 1997 and Kitts *et al.*, 2000). Several of these substances are believed to have potential value as cancer chemo-preventive or therapeutic agents within the human body.

Solanum nigrum (SNL) is a common herb that grows wildly and abundantly in open fields. It has been used in traditional folk medicine because of its diuretic and antipyretic effects. In particular, it has been used to cure inflammation, edema, mastitis and hepatic cancer for a long time ago in oriental medicine (Sultana *et al.*, 1995 and Prashanth *et al.*, 2001). The fruit extract of SNL has been reported to have a strong, dose-dependent cytotoxicity and the ability to induce significant DNA damage in human lymphocytes (Yen *et al.*, 2001). It also has significant hydroxyl radical scavenging potential (Prashanth *et al.*, 2001). SNL contains steroidal glycosides, steroidal alkaloids, steroidal oligoglycosides, solamargine and solasonine (Saijo *et al.*, 1982).

Several studies have been conducted to investigate the nutritive and medicinal value of the 'vegetable black nightshades'; it is evident that these species constitute nutritious vegetables. The leaves can provide appreciable amounts of protein and amino acids, minerals including calcium, iron and phosphorus, vitamins A and C, fat and fiber, as well as appreciable amounts of methionine, an amino acid scarce in other vegetables (Fortuin and Omta, 1980 and FAO, 1988). Moreover the berries can apparently yield high amounts of iron, calcium

and vitamin B (Fortuin and Omta, 1980), and appreciable amounts of vitamin C and carotene. The seeds too contain vitamin C and carotene (Watt and Breyer-Brandwijk, 1962).

Various parts of many of the species belonging to the section *Solanum* are widely used medicinally throughout the world. Their use as such is recorded from the earliest times and various species, especially *Solanum nigrum*, are mentioned and often illustrated in all of the early Herbals, with Dioscorides being one of the first to record their medicinal properties. Since then this 'species' has continued to be widely acclaimed for its medicinal effects in every country in which the taxon is found. The bruised fresh leaves used externally are reputed to ease pain and reduce inflammation; they are applied to burns and ulcers by the Arabs. Leaf juice has also been used for ringworm, gout and earache, while it is also reputed to be a good gargle and mouthwash when mixed with vinegar (Grieve, 1931).

In Japan Saijo *et al.* (1982) observed that immature fruits of *Solanum nigrum* contain steroidal glycosides which show considerable anticancer activity. These glycosides could be solasonine, solamargine, diosgenin and solasodine.

In East Africa the raw fruit is chewed and swallowed for treatment of stomach ulcers or for general abdominal upsets which lead to continued stomach-ache. Infusions of leaves and seeds are rubbed onto the gums of children who have developed crooked teeth. Pounded leaves are soaked in water, fermented and used for the treatment of boils, ulcers and swollen glands. Unripe berries are used to treat ring

worms. Various parts of the plant are also believed to cure malaria, black fever, dysentery and urinary infection (Kokwaro, 1976).

The present investigation was undertaken to detect the presence of some fruit extract secondary metabolites and evaluate their activity as antioxidant, anticancer and hepatoprotectant.