

Therapeutic effectiveness of *Ocimum basilicum* extract on bovine cutaneous papillomatosis**AbdulRahman A. Saied^{1,2*}, Ashraf A. El-Ghoneimy¹, Abdel-latif Seddek³, Sary Kh. Abdel-Ghafar⁴, Samy A.F. Morad¹**

¹ Department of Pharmacology, Faculty of Veterinary Medicine, South Valley University, Qena, 83523, Egypt. ² Aswan Office, Touristic Activities and Interior Offices Sector, Ministry of Tourism and Antiquities, Aswan 81511, Egypt. ³ Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, South Valley University, Qena, 83523, Egypt. ⁴ Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut, 71526, Egypt.

Abstract

Bovine cutaneous papillomatosis is a common cutaneous disease of cattle in the Egyptian veterinary field. *Ocimum basilicum* L. (Rehan) is one of the aromatic plants originating in Asia and Africa. Many studies showed that *Ocimum basilicum* has interesting antiviral and anticancer activities. However, there is no report demonstrating the clinical significance of the anti-papilloma activity of *Ocimum basilicum* against bovine skin papillomas. Thus, our study was designed to evaluate the therapeutic potential of *Ocimum basilicum* extract (OBEx) as an anti-papilloma agent against bovine papillomatosis. OBEx was prepared and undergone phytochemical analysis that revealed presence of alkaloids, phenolics, and flavonoids. Ten cutaneous papillomatosis-infected cattle were diagnosed clinically and histopathologically. Animals were treated with OBEx ointment 2% that topically applied daily and papillomas regression was recorded weekly. Clinically, papillomas started to disappear from the 7th - 21st day after the start of treatment. Histopathological analysis showed improvement in histological features of wart tissue returning to the normal skin structure with presence of lymphocytic infiltration. We concluded that the topical application of OBEx is an effective, promising alternative, cheap, and easily apply agent for treatment of skin papilloma.

Keywords: Basil leaf (*Ocimum basilicum*); Clinicopathological; Bovine cutaneous papillomatosis; Effectiveness

DOI: 10.21608/svu.2020.35596.1067 **Received:** July 13, 2020 **Accepted:** September 12, 2020

Published: September 16, 2020.

***Corresponding Author:** AbdulRahman A. Saied. **E-mail:** saied_abdelrahman@yahoo.com

Citation: Saied et al., 2020, Therapeutic effectiveness of *Ocimum basilicum* extract on bovine cutaneous papillomatosis. SVU-IJVS 2020, 3(2): 60-77.

Copyright: © Saied. This is an open access article distributed under the terms of the creative common attribution license, which permits unrestricted use, distribution and reproduction in any medium provided the original author and source are created.

Competing interest: The authors have declared that no competing interest exists.



INTRODUCTION

Cutaneous papillomatosis is a worldwide viral skin disease caused by the papillomavirus that's more common in cattle than any other domestic animals (Börkú et al., 2007). It is characterized clinically by epithelial hyperplasia and proliferation of connective tissue. It significantly reduces animal welfare and could cause significant financial losses in animal husbandry as lowering milk and meat yields, reducing hide quality, and the unsightly appearance. Bovine papillomavirus (BPV) affects all ages but mostly young cattle (Nicholls and Stanley, 2000 and Özsoy et al., 2011).

Cattle are the source of infection, and the route of infection mostly through both direct and indirect contact, and BPV enters the body through micro trauma, scratches or other skin defects. Fomites, injections, malnutrition, hormonal imbalances, and long-term exposure to sunlight could be risk factors. Papillomas in healthy cattle normally regress after one year, some do not, but may spread to other sites in chronically immunosuppressed animals (Campo, 2006) and may persist, and transformed into malignant neoplasia. Some owners exclude papillomatosis-infected animals for show reasons or for the dysfunction of affected body parts (teats, prepuce, around the vagina, and near the eyes). So, warts may be removed for cosmetic reasons or function restoring of body parts.

Although PVs are species-specific, natural cross-species transmission could occur between *Bos taurus* and other animals as horses (Nasir and Campo, 2008), buffaloes (Silvestre et al., 2009; Pangty et al., 2010; Somvanshi, 2011), cats (Munday and Knight, 2010), African lions (Orbell et al., 2011), Cape mountain zebras, yaks, tapirs, giraffes, and sable

antelopes (Van Dyk et al., 2011 and Williams et al., 2011). Most therapeutic and surgical interventions have limited efficacy in the treatment of bovine or equine papillomatosis (Finlay, 2011).

Ocimum basilicum L. (sweet basil) belongs to the family Lamiaceae, distributed throughout the tropical and subtropical regions of Asia, Africa, and Central and South America (Kintzios and Makri, 2007). Phytoconstituents in basil are polyphenols, flavonoids, and terpenes etc. (Lee and Scagel, 2009) and the plant is widely used in food, pharmaceutical, cosmetic, aromatherapy, and perfumery industries (Aburjai and Natsheh, 2003; Loughrin and Kasperbauer, 2003; Padalia et al., 2017). *O. basilicum* leaves extract has antioxidant, anti-inflammatory (Neergheen et al., 2010), antiviral, and antimicrobial activities (Chiang et al., 2005 and Ahmad et al., 2013). Its extract showed anticancer and anti-proliferative activity against HeLa (Cervical) and HEp-2 (Laryngeal) cancer cell lines (Kathirvel and Ravi, 2012), and it could act as a transdermal enhancer (Fang et al., 2004). Some plant extracts are effective in treating cancer, whose action is attributed to additional or synergistic effect of compounds present in the extract (Li et al., 2000). In consequence, the cytostatic effect observed in tumor cells seems to be more effective than the effect of isolated and biologically active compounds (Vickers, 2002).

The treatment of cutaneous bovine papillomatosis has been remaining a matter of great concern owing to the recurrence of the disease and the difficulties to cure it. However, the focus is still on topical treatment of cutaneous papillomatosis especially medicinal plants which represent an important side in African societies (Olaleye et al., 2008). Due to the

urgent need to find out anti-papilloma agents that are both effective and cheaper, this study aimed to evaluate the effectiveness of *Ocimum basilicum* leaves extract by clinical trials on cutaneous papillomatosis-infected cattle. Therapeutic effectiveness of the extract was clinicopathologically assessed. Hematobiochemical parameters (Palanivel et al., 2017 and Bassi et al., 2019) in addition to the oxidative stress biomarkers were assessed in the blood of infected animals to screen any change in disease-associated body condition and if there is any adverse effect could occur. Infected animals were diagnosed by clinical and histopathological methods.

MATERIALS AND METHODS

Chemicals and Reagents

Hematoxylin-eosin stain and paraffin wax were kindly provided from Department of Pathology, Faculty of Veterinary Medicine, South Valley University. Ethanol was purchased from local suppliers. MDA, SOD, GSH, and GSH-Px Kits were procured from Biodiagnostic (Diagnostic and Research Reagents) Company, Dokki, Giza, Egypt. All other chemicals and solvents were purchased locally and of analytical grade.

Plant materials

The commercial dried and chopped leaves of *Ocimum basilicum* L. (Sweet Basil) were purchased from a local herbal medicine supplier at the tourism market in Aswan city (latitude 24°5'15'' N; longitude 32°53'56'' E), the southern part of Egypt. Leaves were manually screened, and good ones are chosen and ground into a fine powder using mortar and pestle then a laboratory mixer. Identification of the plant was confirmed by Department of Botany, Faculty of Science, South Valley University. Voucher specimens were kept

in the Department of Pharmacology, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt for future reference.

Extract Preparation

In this study, we focused our work on the ethanolic leaf extract of *Ocimum basilicum* (OBEx). Dried material was extracted by maceration with 70% ethanol in a flask for three days at room temperature with periodic stirring, and the flask was closed with aluminum paper and parafilm to prevent evaporation of the solvent in the extraction process. Extraction process was conducted in a place without light to prevent chemical changes in the ingredients of the plant. After 72 hours this mixture was filtered with Whatman filter paper. Filtrate was vacuum dried using rotary evaporator (Heidolph, Germany) to remove the ethanol and taken to complete dryness to obtain the crude extract with a yield of 5% with dark brown color, then kept under refrigeration at 4°C.

Phytochemical screening

Ocimum basilicum leaves ethanolic extract submitted to phytochemical analysis. Different assays were performed qualitatively to detect the presence of alkaloids, glycosides, flavonoids, anthraquinones, saponins, steroids, tannins, and phenolic compounds.

Preparations of OBEx ointment 2%

Topical formulation was prepared, 2% (w/w) 20mg/g ointment formulation of ethanolic extract of leaves and white petroleum jelly was used as the ointment base. The medicated ointment was packed in plastic bottles with closely fitted screw caps, labeled and stored at 25°C pending further use (Figure 1). OBEx ointment was

visually evaluated. It was homogenized with the normal basil aroma and brownish color. No irritation revealed when applied to human skin.



Figure 1: *Ocimum basilicum* ethanolic extract ointment 2% characterizing by homogenized formulation, dark brownish color, and aroma smell.

Field trials and Animals

A total of ten Baladi cattle *Bos taurus* from Qena province exhibiting cutaneous papillomatosis were randomly selected and included in the treatment regime in this study. The age of animals ranged from 1 to 3.5 years (average age: 2.15 years) and weighed 150 – 260 Kg with free access to food and water *ad libitum*. All animals were diagnosed clinically and confirmed histopathologically. Animal sex, age, and weight, and papillomas size, shape, and location were recorded. Head (Face - Ear) and neck regions were the most infected sites in the animal's body.

Animals were treated topically with *Ocimum basilicum* ethanolic extract (OBEx) ointment 2% twice daily by the farmer during the 90-days experiment. Effectiveness was determined by the warts count reduction test and histopathological findings. All efforts were made to minimize both the number of animals used and unwanted stress or discomfort to the animals throughout the experiment period.

Clinicopathological evaluation

The start day of treatment considered as day 0 and the animals were monitored weekly up to 3 months and the regressing process of the papilloma was recorded. The size and shape of papillomas before and after treatment application were compared and the alterations were recorded. Counting of regressed warts over time elapsed until recovery was used to measure the therapeutic efficacy of the extract.

Lesions were biopsied before and after treatment application from the lesion to the level of the dermis perpendicular to the skin surface. Specimens were then collected and fixed in 10% buffered formalin. Warts excision and collection was done by veterinary surgeon and in compliance with international ethical standards for animal welfare. Specimens were dehydrated, cleared, and embedded in paraffin then sectioned at a thickness of 5 μm . Sections were subsequently stained with hematoxylin and eosin (H & E; (Bancroft and Gamble, 2008)). The slides were then observed and analyzed under the light microscope. Post-treated regressed papillomas were collected to monitor the therapeutic effectiveness of topical extract.

Hematobiochemical analysis

Blood obtained from the jugular vein of the animals twice, pre- and post-treatment. The first blood samples served as infected animals, while the second blood samples were considered post-treatment changes. EDTA-containing tubes for CBC (RBCs, Hb concentration, Packed cell volume, Total leucocytes count, Lymphocytes, and Monocytes) and all hematological measurements were performed with a hematology analyzer (Feldman, 2000). Plain tubes for biochemistry; serum samples were used to measure the activities of alanine aminotransferase (ALT), aspartate

aminotransferase (AST), blood urea, and creatinine using automated biochemistry analyzer.

Oxidative stress biomarkers analysis

MDA, GSH, GSH-Px, and SOD (Paksoy et al., 2015) were estimated in the plasma and erythrocytes of papillomatosis-infected animals before and after treatment. Blood collected in EDTA-containing tubes and centrifuged at 1000 rpm for 15 min, to separate plasma and erythrocytes for biochemical assays and stored at -70°C for processing. After centrifugation, the buffy coat was removed, and the plasma and erythrocytes were washed with physiological saline. The erythrocytes were hemolysed by rigorous vortexing. SOD (Nishikimi et al., 1972), GSH-Px (Paglia et al., 1967), GSH, and MDA (Esterbauer et al., 1982) were measured and all procedures were performed according to the manufacturers' instructions. All four parameters were measured spectrophotometrically (Spectrophotometer, Shanghai Aucy Scientific Instrument Co., Ltd., China) in the laboratory of Physiology Department, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt.

Determination of Malondialdehyde (MDA)

0.2 mL of plasma with 1 mL chromogen were mixed well, test tube covered with glass bead and then put in a hot water bath for 30 min. After cooling, the optical densities were measured spectrophotometrically at 534 nm. The changes in MDA levels in plasma samples were measured spectrophotometrically and the results were expressed as nmol MDA/ml.

Determination of Superoxide dismutase SOD

After centrifugation at 3000 rpm for 10 min, the plasma and buffy coat were removed to harvest the red blood cells (RBC), then washing erythrocytes four times with 3ml normal saline, centrifuged at 4000 rpm for 10 min after each wash. The washed centrifuge erythrocytes made up to 2ml with cold redistilled water, mixed and left to stand at +4°C for 15 min and the lysate stored at -70 °C for estimation of SOD level. Working agent was prepared; 10ml phosphate buffer pH=8.5, 1ml nitroblue tetrazolium (NBT) and 1ml NADH. 1ml of working agent and 0.1ml of lysate were mixed well then 0.1ml of phenazine methosulphate (PMS) is added to initiate the reaction. Superoxide dismutase (SOD) activity in hemolysate was measured spectrophotometrically at 560 nm by using nitro blue tetrazolium as a substrate after suitable dilution. SOD activity was expressed in Unit/ml.

Determination of Glutathione peroxidase GSH-Px

Glutathione peroxidase activity in the sample was measured according to Paglia and Valentine's method (Paglia et al., 1967). The activity of GSH-Px was measured at 340 nm by measuring the decrease of NADPH absorbance using an extension coefficient of 6.22 mM⁻¹ cm⁻¹ and the results were expressed as mU/ml. After centrifugation at 4000 rpm for 10 min, the plasma and buffy coat were removed to harvest the red blood cells (RBC), then washing erythrocytes three times with four volumes normal saline, red cell pellet is lysed by adding four volumes of cold distilled water to the estimated volume, centrifuging at 4000 rpm for 10 min for collecting supernatant then stored at -70°C for estimation of GSH-Px level.

Determination of reduced glutathione (GSH)

After centrifugation at 4000 rpm for 10 min, the plasma and buffy coat were removed to harvest the red blood cells (RBC), then washing erythrocytes three times with four volumes normal saline, red cell pellet is lysed by adding four volumes of cold distilled water to the estimated volume, centrifuging at 4000 rpm for 10 min for collecting supernatant then stored at -70°C for estimation of GSH level (Beutler, 1963). 0.1ml of lysate, 0.5ml distilled water and 0.5ml trichloroacetic acid (TCA) were mixed well, allowed to stand for 5 min, centrifuge at 3000 rpm for 15 min then 0.5ml of supernatant, 1ml buffer and 0.1ml DTNB were mixed well-measured after 5- 10 min at 405 nm and the results were expressed as mg/dl.

Data analysis

Data of the treated groups were compared with the same animals before treatment to determine the significance of treatment efficacy. Statistical analysis was performed by the computer program SPSS/PC (2001) using one-way ANOVA test for calculating means and standard deviations. Statistical significance was assumed at the $P < 0.05$ level.

Ethics statement

Papillomatosis-infected animals were randomly selected in this study after approval of the animal owners; Oral consent was obtained from all the animal owners for sampling and picturing. All efforts were made to minimize any suffering for the animals and all procedures were performed and approved by Animal Ethics Committee at South Valley University, Qena, Egypt.

RESULTS

Plant Material and Phytochemical screening

The ethnobotanical data of *Ocimum basilicum* and its extract percentage yield is illustrated in Table 1. The yield of the extract was 5% w/w, dark brown in color and insoluble in water.

Table 1: The ethnobotanical data of *Ocimum basilicum* extract

Plant species	<i>Ocimum basilicum</i>
Family	Lamiaceae
Local name	Rehan
Common name	Basil
Plant part used	Leaves
Extract pH	5.6
Extract yield (%)	5

The phytochemical screening (Table 2) revealed that *Ocimum basilicum* leaves contains alkaloids, glycosides, anthraquinones, phenolics, saponins, flavonoids, tannins, and steroids.

Table 2: Phytochemical screening of ethanol extract of *Ocimum basilicum*

S. No	Phytochemical Test	Extract
1	Alkaloids	+
2	Glycosides	+
3	Saponins	+
4	Phenolics	+
5	Steroids	+
6	Flavonoids	+
7	Tannins	+
8	Anthraquinones	+

+ = Present - = absent

Clinicopathological findings

Clinically, warts sizes, shapes, locations, and numbers were precisely recorded and clinically examined as well as animal age, sex, and weight. Warts regression and time elapsed until recovery of the infected animals was used to measure the clinical efficiency of extract.

Macroscopically, warts were 0.5 to 15 cm in size, reddish, grey-white, or dark

in color, solitary or multiple, cauliflower-like shape, sessile or pedunculated, the lesions surface is semi soft, rough, or dry. The lesions appeared on different parts of the animal's body; face, around eyes, legs, and neck (Figure 2a, b, and c). The excised tumors were lobulated, and their cut surface reveals an external layer of keratinized epithelium and an internal core of homogeneous white connective tissue.



Figure 2 showing (2a): Cauliflower-like papilloma with grey-white color and its surface is dry. It appeared in the neck with diameter about 4.5 cm. (2b): Cauliflower-like and lobulated papilloma with dark color and its surface is tough. It appeared in the leg with diameter about 3.5 cm. (2c): Cauliflower-like and lobulated papilloma with reddish color. It appeared in the leg with diameter about 3.5 cm

Papillomas reduced in size from the 7th – 21st day after the start of treatment, while the complete recovery was noticed (all papillomas were completely regressed) within 28th - 84th day after the start of treatment (Figure 3 – 4 a, b, c, and d) with average number of days about 54 days from start of treatment till complete recovery.

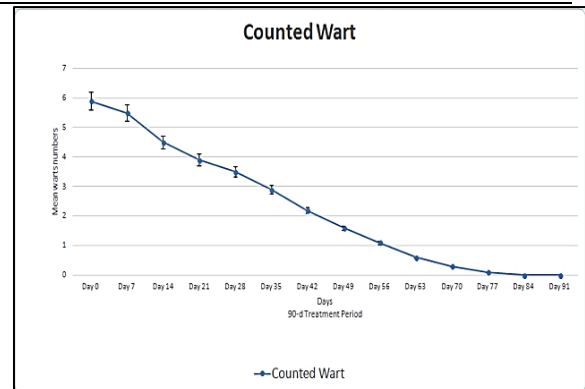


Figure 3: Mean papillomas score after 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 84, and 91 days post treatment. Papillomas scale represented as reduction in papillomas number.



Figure 4 showing (4a): Pre-treatment lesion showed cauliflower-like and dry surface with grayish-white in color. (4b): After 14 days, lesion showed color paleness with appearance of small projections on its surface. (4c): After 21 days, lesion showed some dryness and paleness in color. (4d): After 56days, skin of infected animal showed complete recovery.

Post-treatment changes in the gross morphology of lesions; some papillomas protrude on the surface (more prominent at the surface than before) as if they increase in size and by the time it becomes easily removed by hand, with rubbing the animal body with other hard objects like walls or spontaneously drop off. In other lesions, we noticed signs of hyperemia (local inflammatory reaction). By the follow up of the treated cases for one year; there was no complaint of recurrence (Figure 5a and b).

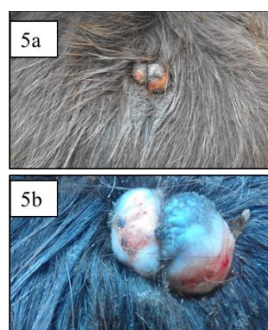


Figure 5 showing (5a): Sizes of two papillomas are increased after 7 days. **(5b):** Protrusion of two papillomas on the surface of skin after 14 days (Plugging of papilloma as it out from its root, hyperemic & soft).

Histopathologically, samples from clinical papillomas-free skin from cattle collected and used as a negative control. Normal bovine skin showed normal epidermis and dermis without any morphological alterations (Figure 6).

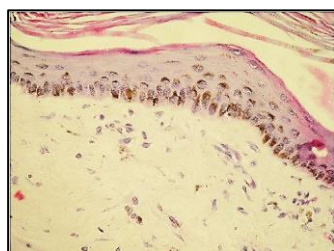


Figure 6: Normal bovine skin section from control animal showing normal histological structure formed of upper epidermal stratified squamous cornified epithelium layer and lower dermal layer formed of connective tissue. H&E stain.10X40

Pre-treatment lesions specimens showed extensive papillomatous growth with severe hyperkeratosis in stratum corneum and severe acanthosis (increase in the thickness of the stratum spinosum of the epidermis) which appeared as finger-like projections (Figure 7a and b).

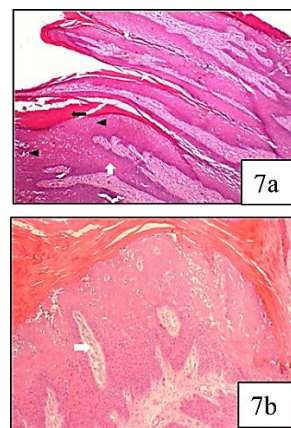


Figure 7 showing (7a): Skin papilloma showed an increase in the thickness of the epidermis (white arrow) with down growth in the dermal layer and contains a core of connective tissue with severe hyperkeratosis (black arrow) and vacuolation of the stratum spinosum (black arrowhead). H&E. 10X 40X. **(7b):** Papilloma characterized with hyperkeratosis and acanthosis with fibrovascular areas (white arrow) and presence of koilocytes. H&E. 10X10

Post-treatment lesions specimens showed improvement in histological features of wart tissue to normal skin structure as decreased epidermal proliferation till be thinner and more increased fibroplasia of connective tissue core (blunt finger-like projection). The dermal layer showed focal and diffuse lymphocytic cell infiltration (Figure 8a and b).

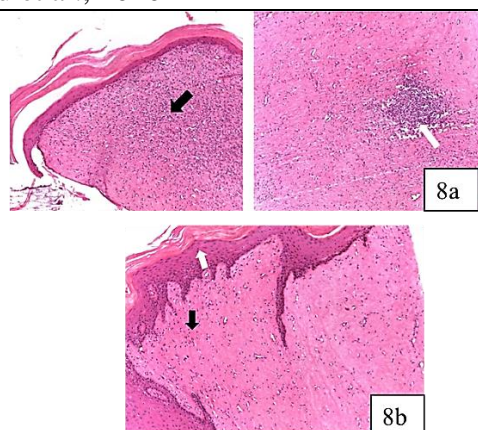


Figure 8 showing (8a): The dermal layer showing diffuse (black arrow) and focal (white arrow) lymphocytic cell infiltration with thinning of epidermal hyperplasia. H&E. 10X. **(8b):** Photomicrograph showing decreased epidermal proliferation till is more thin (white arrow) and increased fibroplasia of connective tissue core (blunt finger like projection) with mild mononuclear cellular infiltration (black arrow). H & E. 40X.

Hematobiochemical findings

Post-treatment hematobiochemical findings (Table 3) in papillomatosis-infected cattle showed a significant decrease in mean PCV and lymphocytes count ($p < 0.05$), while monocytes showed a significant increase ($p < 0.05$). AST showed a significant ($P < 0.05$) decrease and Urea level displayed a significant decrease. Creatinine level showed a non-significant decrease. Clinical biochemistry is mainly performed to evaluate the effect of drugs on hepatic and renal function where the liver is the major site for the metabolism of drugs. The transaminases AST and ALT are the enzymes that play an important role in liver function and have been used as biomarkers for predicting possible toxicity (Tietz et al., 2008).

Table 3: The mean \pm SE of some haemato-biochemical parameters (RBCs, haemoglobin, PCV %, TLC, lymphocytes %, monocytes, AST, ALT, BUN, and creatinine) in cutaneous papillomatosis-infected animals treated with topical *Ocimum basilicum* extract.

Parameter	Hematologic parameters	
	Before	After
RBC's ($\times 10^6/\text{mm}^3$)	9.36 \pm 0.32	8.5 \pm 0.1
Hb. (gm/dl)	10.04 \pm 0.29	9.84 \pm 0.13
PCV (%)	34.59 \pm 0.95	32.39 \pm 0.17*
TLC ($\times 10^3/\text{mm}^3$)	10.73 \pm 0.09	10.36 \pm 0.12
Lymphocytes %	78.75 \pm 1.05	75.52 \pm 0.95*
Monocytes %	9.92 \pm 1.23	19.55 \pm 0.98*
Parameter	Biochemical parameters	
	Before	After
AST (IU/l)	60.2 \pm 2.35	55.03 \pm 0.23*
ALT (IU/l)	29.6 \pm 0.31	29.1 \pm 0.18
BUN (mg/dl)	27.6 \pm 1.63	18.4 \pm 2.45*
Creatinine (mg/dl)	1.34 \pm 0.07	1.19 \pm 0.05

* \rightarrow referring to significant difference when compared with before and after within the same group when $P < 0.05$.

Oxidative Status

Post-treatment, MDA activity significantly decreased, while GSH-Px activity significantly increased as shown in Table 4. While a non-significant decrease in SOD and GSH activities were recorded.

Table 4: The mean \pm SE of some Oxidative stress markers (MDA, SOD, GSH-Px, GSH) in cutaneous papillomatosis-infected animals treated with topical *Ocimum basilicum* extract.

Parameter	Oxidative stress biomarkers	
	Before	After
MDA (n mole/L)	6.26 \pm 0.08	2.83 \pm 0.02*
SOD (U/ml)	3.25 \pm 0.02	2.95 \pm 0.04
GSH-Px (mU/ml)	33.61 \pm 1	45.83 \pm 2.2*
GSH (mg/dl)	5.31 \pm 0.19	5.11 \pm 0.02

* \rightarrow referring to significant difference when compared with before and after within the same group when $P < 0.05$.

DISCUSSION

Medicinal plants and their bioactive compounds have been tested on many skin cancer cell lines and animal models

showing promising anti-skin cancer activities as chemopreventive and chemotherapeutic agents (Dhandevi and Jeewon, 2015) by inhibiting cancer cell development and progression (Penta et al., 2018).

Ocimum basilicum acts as anti-inflammatory (Raina et al., 2016), antioxidant (Flanigan and Niemeyer, 2014 and Pandey et al., 2016), anti-microbial (Suppakul et al., 2003 and Nguyen and Niemeyer, 2008), anti-fungal (Oxenham et al., 2005 and Kumar et al., 2011), insecticidal (Freire et al., 2006 and Kathirvel and Ravi, 2012), immunomodulatory (Dashputre and Naikwade, 2010) and cytotoxicity agent (Aarthi and Murugan, 2010) thanks to its promising phytoconstituents.

The antioxidative capacity of flavonoids confers a therapeutic potential on cancer (Yang et al., 2000), viral diseases, and inflammations (Nijveldt et al., 2001 and Nair et al., 2006). The different components of OB are used as remedies for treating disorders such as viral ophthalmic, respiratory, and hepatic infections (Chiang et al., 2005; Yacout et al., 2012; Eftekhar et al., 2019). Ethanolic extract of *Ocimum basilicum* yielded apigenin, linalool, and ursolic acid exhibiting a broad spectrum of antiviral activities (Chiang et al., 2005) also camphor and 1,8-cineole against bovine viral disease virus BVDV (Kubiça et al., 2014).

The antitumor activity of OB in mice (Novotný et al., 2001 and Dasgupta et al., 2004) may due to d-limonene (Amanzadeh et al., 2006) or eugenol (Jaganathan and Supriyanto, 2012), or both by induction of apoptosis. Roles of ursolic acid UA and eugenol in basil against skin papillomas and cancers were intensively reported (Iqbal et al., 2019) besides their other

biological properties. *Ocimum basilicum* leaves extracts rich in flavonoids which can prevent or treat cutaneous inflammation and malignancy by maintaining the skin's homeostasis (Cijo George et al., 2016). Studies showed the antitumor activity of *Ocimum basilicum* against HeLa (Kathirvel and Ravi, 2012) and Hep 2 cell lines (Manosroi et al., 2006). OB has been shown to reduce the risk of skin and fore stomach papillomagenesis (Dasgupta et al., 2004). Aswan province (Abd El-Azim et al., 2015) is one of the major cultivation centers for the aromatic and medicinal plants e.g. *Ocimum basilicum* in Upper Egypt (El-Demerdash, 2001).

BPV is an ubiquitous virus that present in all continents (He et al., 2016). BPV detected in the blood of healthy and papillomatosis-affected cattle (Silva et al., 2013) and could be vertically transmitted. The economic impact of bovine papillomatosis force us for seek for finding out an anti-papilloma agent.

Post-treatment clinical evaluation showed a regression of papillomas on 7th - 21st day after the start of treatment indicating the direct anti-proliferative action of extract. The healed animals became free of cutaneous papillomatosis without recurrence for one year. Post-treatment pathological studies revealed that the finger-like projections associated with severe acanthosis of wart tissue changed into normal skin structure as decreased epidermal proliferation till be thinner and more increased fibroplasia of connective tissue core (blunt finger-like projection). Presence of focal and diffuse lymphocytic cell infiltration in the dermal layer suggested the immunostimulant action of extract by recruitment of lymphocytes, the main immunity cells for this disease at the site of infection.

The interesting in cutaneous papillomatosis that the hematological parameters of BPV-infected animals negatively changed as a significant reduction of hemoglobin, packed cell volume (PCV), and hematocrit, and produced leucopenia with lymphopenia and monocytopenia suggesting disease-induced stress releasing endogenous corticosteroids in response to (Palanivel et al., 2017 and El-Mandrawy and Alam, 2018) or due to weakening of animals, off food, or anemia (Palanivel et al., 2017 and Bassi et al., 2019). A significant ($P < 0.05$) increase in monocytes was recorded while, PCV% and lymphocytes significantly decreased. The transient fall in the values of these indices may be due to the uncontrolled stress during the study.

The liver is one of the body organs which responsible for destructing and detoxification all ingested xenobiotics (Adeoye et al., 2004) and the kidney which responsible for elimination of many xenobiotics and prescription drugs (Bajaj et al., 2018). The beneficial role of ethanolic OB leaves extract on hepatic antioxidant defense enzymes (which correlate with attenuating the risk of chemical carcinogenesis) and its therapeutic effect on skin cancer and stomach cancer was reported (Dasgupta et al., 2004). The hepatoprotective effects of *Ocimum basilicum* (sweet basil) extracts may be linked to their antioxidant activities (Gbadegesin and Odunola, 2010). BPV infection significantly increased serum AST activity, a phenomenon that reflects hepatocyte damage, even if such damage is subclinical (Barakat et al., 2013). Besides, AST is present in cardiac and skeletal muscle cells. In the current study, a significant decrease in AST activity and an insignificant decrease ALT activity when compared with the values of the same group before treatment indicating the

possibility of the extract having some degree of hepatoprotective effect and non-harmful effect on the kidney, that may be attributed to their phytoconstituents (flavonoids, saponins) (Pan et al., 2006 and Roy et al., 2006) and antioxidant activity. Creatinine values insignificantly reduced.

Lipid peroxidation was reported in cattle with cutaneous papillomatosis (Aslan and Saraç, 2011 and Paksoy et al., 2015) with increase of plasma MDA and decrease in erythrocyte SOD. A few studies link blood antioxidant and lipid peroxidation status to the skin diseases of cattle (El-Mandrawy and Alam, 2018). Oxidative stress increases the production of oxidants, such as MDA, that can influence the release of proinflammatory mediators, such as cytokines; these mediators play important roles in the induction of inflammation in certain skin diseases (Johnson-Huang et al., 2009) and suppression of T lymphocytes (Aslan and Saraç, 2011). So, decreasing the oxidative stress and enhancing the antioxidant capacity used as a line in treatment of bovine papillomatosis.

The same situation in HPV-patients which antioxidant enzymes were analyzed in the erythrocytes and plasma of human peripheral blood (Manju et al., 2002) and revealed low levels of GSH, GSH-Px, GST, and SOD in the circulation of cervical cancer patients that's they were extensively utilized to scavenge lipid peroxides as well their sequestration by tumor cells (Kim et al., 2004; Beevi et al., 2007; Nirmala and Narendhirakannan, 2011). The antioxidant enzyme activities of erythrocyte in the papillomatosis infected-animals can be used as peripheral evidence on oxidative stress besides that erythrocytes are easy to obtain (Sasmaz et al., 2005). So, we have chosen erythrocyte (Palanivel et al., 2017 and Bassi et al.,

2019) to estimate the antioxidant enzyme activities in BPV-patients with cutaneous papillomatosis. A significant decreased MDA (lipid peroxidation) and a significant increase in GSH-Px were observed while changes in SOD and GSH activities were insignificant. Decreasing in the general oxidative status, as a peripheral response, may be associated with cutaneous papillomas regressions and extract ingredients.

CONCLUSIONS

OBEx ointment-treated animals showed clinical and histopathological improvements with no side effects and no recurrence were observed. Papillomas regression might be attributed due to the immunostimulant, direct anti-proliferative action, and antioxidant activity of extract thanks to its potent active ingredients. Thus, we concluded that topical ointment from *Ocimum basilicum* ethanolic extract (OBEx) is a probably cost-effective and promising drug for treatment of bovine cutaneous papillomatosis in Egypt with considerable safety and considerable economic benefit to the farmers.

ACKNOWLEDGEMENTS

We would to thank Dr. Asmaa A. Metwally, Department of Surgery, Anesthesiology, and Radiology, Faculty of Veterinary Medicine, Aswan University, Egypt for her invaluable help in clinical studies, sampling, and picturing; Dr. Hassan Ahmed, Physiology Department, Faculty of Veterinary Medicine, South Valley University, for his kind help in carrying out antioxidant assays. We also extend special thanks to cattle owners and field veterinarians for their co-operation on trial days.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

- Aarhi, N. & Murugan, K. (2010). Larvicidal and repellent activity of *Vetiveria zizanioides* L, *Ocimum basilicum* Linn and the microbial pesticide spinosad against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Journal of Biopesticides*, 3(Special Issue): 199.
- Abd El-Azim, M. H., Abdelgawad, A. A., El-Gerby, M., Ali, S. & El-Mesallamy, A. M. (2015). Phenolic compounds and cytotoxic activities of methanol extract of basil (*Ocimum basilicum* L.). *J Microb Biochem Technol*, 7(4): 182-185.
- Aburjai, T. & Natsheh, F. M. (2003). Plants used in cosmetics. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 17(9): 987-1000.
- Adeoye, B. & Oyedapo, O. (2004). Toxicity Of *Erythrophleum Guineense*. *African Journal of Traditional, Complementary and Alternative Medicines*, 1(1): 45-54.
- Ahmad, N., Fazal, H., Abbasi, B. H., Anwar, S. & Basir, A. (2013). DPPH free radical scavenging activity and phenotypic difference in hepatoprotective plant (*Silybum marianum* L.). *Toxicology and industrial health*, 29(5): 460-467.
- Amanzadeh, Y., Ashrafi, M. & Mohammadi, F. (2006). New elaborated technique for isolation and purification of limonene from

- orange oil. *Iranian Journal of Pharmaceutical Sciences*, 2(2): 87-90.
- Aslan, Ö. & Saraç, Ş. (2011). Lipid peroxidation level and antioxidant enzyme activities in bovine papillomatosis. *Erciyes Üniversitesi Veteriner Fakültesi Dergisi*, 8(2): 75-81.
- Bajaj, P., Chowdhury, S. K., Yucha, R., Kelly, E. J. & Xiao, G. (2018). Emerging kidney models to investigate metabolism, transport, and toxicity of drugs and xenobiotics. *Drug Metabolism and Disposition*, 46(11): 1692-1702.
- Bancroft, J. D. & Gamble, M. (2008). *Theory and practice of histological techniques*, 6th Edition, Churchill Livingstone, Elsevier health sciences, China.
- Barakat, S. E. M., Alhizab, F. A. & Elbahr, S. M. (2013). Clinicopathological and serobiochemical investigation of naturally occurring cutaneous papillomatosis in dromedary camels (*Camelus dromedarius*). *Sci Int*, 1: 212-216.
- Bassi, P. B., Araujo, F. F., Garcia, G. C., E Silva, M. F. C., Bittar, E. R., Bertonha, C. M., Martins-Filho, O. A., Araujo, M. S. S. & Bittar, J. F. (2019). Haematological and immunophenotypic evaluation of peripheral blood cells of cattle naturally infected with bovine papillomavirus. *The Veterinary Journal*, 244: 112-115.
- Beevi, S. S., Rasheed, M. H. & Geetha, A. (2007). Evidence of oxidative and nitrosative stress in patients with cervical squamous cell carcinoma. *Clin Chim Acta*, 375: 119-23.
- Beutler, E. (1963). Improved method for the determination of blood glutathione. *J. lab. clin. Med.*, 61: 882-888.
- Börkücü, M., Atalay, O., Kibar, M., Çam, Y. & Atasever, A. (2007). Ivermectin is an effective treatment for bovine cutaneous papillomatosis. *Research in veterinary Science*, 83(3): 360-363.
- Campo, M. S. (2006). *Papillomavirus research: from natural history to vaccines and beyond*, Caister Academic Press; Norfolk. *Bovine papillomavirus: Old system, new lessons?* 373-387
- Chiang, L. C., Ng, L. T., Cheng, P. W., Chiang, W. & Lin, C. C. (2005). Antiviral activities of extracts and selected pure constituents of *Ocimum basilicum*. *Clinical and Experimental Pharmacology and Physiology*, 32 (10): 811-816.
- Cijo George, V., Vijayakumaran Vijesh, V., Inoka Madumani Amararathna, D., Ayshwarya Lakshmi, C., Anbarasu, K., Ragupathi Naveen Kumar, D., Radhakrishnan Ethiraj, K., Ashok Kumar, R. & P Vasantha Rupasinghe, H. (2016). Mechanism of action of flavonoids in prevention of inflammation-associated skin cancer. *Current medicinal chemistry*, 23(32): 3697-3716.
- Dasgupta, T., Rao, A. & Yadava, P. (2004). Chemomodulatory efficacy of basil leaf (*Ocimum basilicum*) on drug metabolizing and antioxidant enzymes, and on carcinogen-induced skin and forestomach papillomagenesis. *Phytomedicine*, 11(2-3): 139-151.

- Dashputre, N. L. & Naikwade, N. S. (2010). Preliminary immunomodulatory activity of aqueous and ethanolic leaves extracts of *Ocimum basilicum* Linn in mice. *International Journal of PharmTech Research*, 2(2): 1342-1349.
- Dhandevi, P. E. M. & Jeewon, R. (2015). Fruit and vegetable intake: Benefits and progress of nutrition education interventions-narrative review article. *Iranian journal of public health*, 44(10): 1309–1321.
- Eftekhar, N., Moghimi, A., Roshan, N. M., Saadat, S. & Boskabady, M. H. (2019). Immunomodulatory and anti-inflammatory effects of hydro-ethanolic extract of *Ocimum basilicum* leaves and its effect on lung pathological changes in an ovalbumin-induced rat model of asthma. *BMC Complementary and Alternative Medicine*, 19(1): 349.
- El-Demerdash, M. (2001). Medicinal plants of Egypt. In : Saxena P.K. (eds) *Development of Plant-Based Medicines: Conservation, Efficacy and Safety* (pp.69-93). Springer, Dordrecht.
- El-Mandrawy, S. A. & Alam, R. T. (2018). Hematological, biochemical and oxidative stress studies of lumpy skin disease virus infection in cattle. *Journal of Applied Animal Research*, 46(1): 1073-1077.
- Esterbauer, H., Cheeseman, K., Dianzani, M., Poli, G. & Slater, T. (1982). Separation and characterization of the aldehydic products of lipid peroxidation stimulated by ADP-Fe²⁺ in rat liver microsomes. *Biochemical Journal*, 208(1): 129-140.
- Fang, J. Y., Leu, Y. L., Hwang, T. L. & Cheng, H. C. (2004). Essential oils from sweet basil (*Ocimum basilicum*) as novel enhancers to accelerate transdermal drug delivery. *Biological and Pharmaceutical Bulletin*, 27(11): 1819-1825.
- Feldman, B. F., Zinkl, J. G., Jain, N. C. & Stein, S. S. (2000). *Schalm's Veterinary Hematology*, 5th ed. Baltimore, MD: Lippincott Williams & Wilkins.
- Finlay, M. (2011). Equine sarcoids and bovine papillomavirus: unravelling the viral pathogenesis. (Doctoral dissertation, University of Glasgow).
- Flanigan, P. M. & Niemeyer, E. D. (2014). Effect of cultivar on phenolic levels, anthocyanin composition, and antioxidant properties in purple basil (*Ocimum basilicum* L.). *Food chemistry*, 164: 518-526.
- Freire, C. M. M., Marques, M. O. M. & Costa, M. (2006). Effects of seasonal variation on the central nervous system activity of *Ocimum gratissimum* L. essential oil. *Journal of Ethnopharmacology*, 105(1-2): 161-166.
- Gbadegehin, M. A. & Odunola, O. A. (2010). Aqueous and ethanolic leaf extracts of *Ocimum basilicum* (sweet basil) protect against sodium arsenite-induced hepatotoxicity in Wistar rats. *Nigerian Journal of Physiological Sciences*, 25(1): 29–36.
- He, Z., Meng, Q., Qiao, J., Peng, Y., Xie, K., Liu, Y., Cai, X., Zhang, J. & Chen, C. (2016). Mixed Nipple Infections Caused by Variant of BPV 3 and a

- Putative New Subtype of BPV in Cattle. *Transboundary emerging diseases*, 63(1): 140-143.
- Iqbal, J., Abbasi, B. A., Ahmad, R., Batool, R., Mahmood, T., Ali, B., Khalil, A. T., Kanwal, S., Shah, S. A., Alam, M. M. & Bashir, S. (2019). Potential phytochemicals in the fight against skin cancer: Current landscape and future perspectives. *Biomedicine & Pharmacotherapy*, 109: 1381-1393.
- Jaganathan, S. K. & Supriyanto, E. (2012). Antiproliferative and molecular mechanism of eugenol-induced apoptosis in cancer cells. *Molecules*, 17(6): 6290-6304.
- Johnson-Huang, L. M., McNutt, N. S., Krueger, J. G. & Lowes, M. A. (2009). Cytokine-producing dendritic cells in the pathogenesis of inflammatory skin diseases. *Journal of clinical immunology*, 29(3): 247-256.
- Kathirvel, P. & Ravi, S. (2012). Chemical composition of the essential oil from basil (*Ocimum basilicum* Linn.) and its in vitro cytotoxicity against HeLa and HEP-2 human cancer cell lines and NIH 3T3 mouse embryonic fibroblasts. *Natural Product Research*, 26(12): 1112-1118.
- Kim, Y. T., Kim, J. W., Choi, J. S., Kim, S. H., Choi, E. K. & Cho, N. H. (2004). Relation between deranged antioxidant system and cervical neoplasia. *International Journal of Gynecologic Cancer*, 14(5): 889-895.
- Kubiça, T. F., Alves, S. H., Weiblen, R. & Lovato, L. T. (2014). In vitro inhibition of the bovine viral diarrhoea virus by the essential oil of *Ocimum basilicum* (basil) and monoterpenes. *Brazilian Journal of Microbiology*, 45(1): 209-214.
- Kumar, A., Shukla, R., Singh, P., Prakash, B., Dubey, N. K. (2011). Chemical composition of *Ocimum basilicum* L. essential oil and its efficacy as a preservative against fungal and aflatoxin contamination of dry fruits. *International journal of food science & technology*, 46(9): 1840-1846.
- Lee, J. & Scagel, C. F. (2009). Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. *Food Chemistry*, 115(2): 650-656.
- Li, X.-K., Motwani, M., Tong, W., Bornmann, W. & Schwartz, G. K. (2000). Huanglian, a Chinese herbal extract, inhibits cell growth by suppressing the expression of cyclin B1 and inhibiting CDC2 kinase activity in human cancer cells. *Molecular Pharmacology*, 58(6): 1287-1293.
- Loughrin, J. H. & Kasperbauer, M. J. (2003). Aroma content of fresh basil (*Ocimum basilicum* L.) leaves is affected by light reflected from colored mulches. *Journal of agricultural and food chemistry*, 51(8): 2272-2276.
- Kintzios, S. & Makri, O. (2008). *Ocimum* sp.(basil): Botany, cultivation, pharmaceutical properties, and biotechnology. *Journal of herbs, spices & medicinal plants*, 13(3): 123-150.
- Manju, V., Sailaja, J. K. & Nalini, N. (2002). Circulating lipid peroxidation and antioxidant status in cervical cancer patients: a case-control study.

- Clinical Biochemistry, 35(8): 621-625.
- Manosroi, J., Dhumtanom, P. & Manosroi, A. (2006). Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer letters*, 235(1): 114-120.
- Munday, J. S. & Knight, C. G. (2010). Amplification of feline sarcoid-associated papillomavirus DNA sequences from bovine skin. *Vet Dermatology*, 21(4), 341-344.
- Nair, M. P., Mahajan, S., Reynolds, J. L., Aalinkeel, R., Nair, H., Schwartz, S. A. & Kandaswami, C. (2006). The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF- κ B system. *Clinical and vaccine immunology*, 13(3): 319-328.
- Nasir, L. & Campo, M. S. (2008). Bovine papillomaviruses: their role in the aetiology of cutaneous tumours of bovids and equids. *Veterinary dermatology*, 19(5), 243-254.
- Neergheen, V. S., Bahorun, T., Taylor, E. W., Jen, L. S. & Aruoma, O. (2010). Targeting specific cell signaling transduction pathways by dietary and medicinal phytochemicals in cancer chemoprevention. *Toxicology* 278(2): 229-241.
- Nguyen, P. M. & Niemeyer, E. D. (2008). Effects of nitrogen fertilization on the phenolic composition and antioxidant properties of basil (*Ocimum basilicum* L.). *Journal of agricultural and food chemistry*, 56(18): 8685-8691.
- Nicholls, P. K. & Stanley, M. A. (2000). The immunology of animal papillomaviruses. *Veterinary immunology and immunopathology*, 73(2), 101-127.
- Nijveldt, R. J., Van Nood, E. L. S., Van Hoorn, D. E., Boelens, P. G., Van Norren, K. & Van Leeuwen, P. A. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *The American journal of clinical nutrition*, 74(4): 418-425.
- Nirmala, J. G. & Narendhirakannan, R. (2011). Detection and genotyping of high-risk HPV and evaluation of anti-oxidant status in cervical carcinoma patients in Tamil Nadu State, India-a case control study. *Asian Pacific Journal of Cancer Prevention*, 12(10): 2689-2695.
- Nishikimi, M., Rao, N. A. & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and biophysical research communications*, 46(2): 849-854.
- Novotný, L., Vachalkova, A. & Biggs, D. (2001). Ursolic acid: an anti-tumorigenic and chemopreventive activity. *Minireview. Neoplasma*, 48(4): 241-246.
- Olaleye, M. T. & Rocha, B. J. (2008). Acetaminophen-induced liver damage in mice: effects of some medicinal plants on the oxidative defense system. *Experimental and Toxicologic Pathology*, 59(5): 319-327.

- Orbell, G. M. B., Young, S. & Munday, J. S. (2011). Cutaneous sarcoids in captive African lions associated with feline sarcoïd-associated papillomavirus infection. *Veterinary pathology*, 48(6), 1176-1179.
- Oxenham, S. K., Svoboda, K. P. & Walters, D. R. (2005). Antifungal activity of the essential oil of basil (*Ocimum basilicum*). *Journal of phytopathology*, 153(3): 174-180.
- Özsoy, Ş. Y., Özyıldız, Z. & Güzel, M. (2011). Clinical, pathological and immunohistochemical findings of bovine cutaneous papillomatosis. *Ankara Üniv Vet Fak Derg*, 58 (3), 161-165,
- Padalia, R. C., Verma, R. S., Upadhyay, R. K., Chauhan, A. & Singh, V. R. (2017). Productivity and essential oil quality assessment of promising accessions of *Ocimum basilicum* L. from north India. *Industrial crops and products*, 97: 79-86.
- Paglia, D. E. & Valentine, W. N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The Journal of laboratory clinical medicine*, 70(1): 158-169.
- Paksoy, Z., Gülesci, N., Kandemir, F. M. & Dinçel, G. Ç. (2015). Effectiveness of levamisole and tarantula cubensis extract in the treatment of teat Papillomatosis of cows. *Indian Journal of Animal Research*, 49(5), pp.704-708.
- Palanivel, K. M., Surendar, A. P. & Kanimozhi, P. (2017). Sero biochemical profile of crossbred calves naturally infected by bovine papilloma virus. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 366-371.
- Pan, L., Xu, L., Zhao, X., Gao, J., Chen, J., Fang, F., Xu, Q. & Tang, X. (2006). Mechanism underlying mitochondrial protection of asiatic acid against hepatotoxicity in mice. *Journal of Pharmacy Pharmacology*, 58(2): 227-233.
- Pandey, V., Patel, A. & Patra, D. D. (2016). Integrated nutrient regimes ameliorate crop productivity, nutritive value, antioxidant activity and volatiles in basil (*Ocimum basilicum* L.). *Industrial Crops Products*, 87: 124-131.
- Pangty, K., Singh, S., Goswami, R., Saikumar, G. & Somvanshi, R. (2010). Detection of BPV-1 and -2 and quantification of BPV-1 by real-time PCR in cutaneous warts in cattle and buffaloes. *Transboundary and emerging diseases*, 57(3), 185-96.
- Penta, D., Somashekar, B. S. & Meeran, S. M. (2018). Epigenetics of skin cancer: Interventions by selected bioactive phytochemicals. *Photodermatology, photoimmunology photomedicine*, 34(1): 42-49.
- Raina, P., Deepak, M., Chandrasekaran, C. V., Agarwal, A., Wagh, N. & Kaul-Ghanekar, R. (2016). Comparative analysis of anti-inflammatory activity of aqueous and methanolic extracts of *Ocimum basilicum* (basil) in RAW264. 7, SW1353 and human primary chondrocytes in respect of the management of osteoarthritis. *Journal of Herbal Medicine*, 6(1): 28-36.

- Roy, C. K., Kamath, J. V. & Asad, M. (2006). Hepatoprotective activity of *Psidium guajava* Linn. leaf extract. *Indian Journal of Experimental Biology*, (44): 305-311
- Sasmaz, S., Arican, O. & Kurutas, E. B. (2005). Oxidative stress in patients with nongenital warts. *Mediators of inflammation*, 4 (2005) 233–236
- Silva, M. A. R., De Albuquerque, B. M. F., Pontes, N. E., Coutinho, L. C. A., Leitão, M. C. G., Reis, M. C., Castro, R. S. & Freitas, A. C. (2013). Detection and expression of bovine papillomavirus in blood of healthy and papillomatosis-affected cattle. *Genet Mol Res*, 12(3): 3150-3156.
- Silvestre, O., Borzacchiello, G., Nava, D., Iovane, G., Russo, V., Vecchio, D., D'ausilio, F., Gault, E. A., Campo, M. S. & Paciello, O. (2009). Bovine papillomavirus type 1 DNA and E5 oncoprotein expression in water buffalo fibropapillomas. *Vet Pathol*, (46), 636-641.
- Somvanshi, R. (2011). Papillomatosis in buffaloes: a less-known disease. *Transboundary and emerging diseases*, 58(4), 327-332.
- Suppakul, P., Miltz, J., Sonneveld, K. & Bigger, S. W. (2003). Antimicrobial properties of basil and its possible application in food packaging. *Journal of agricultural and food chemistry*, 51(11): 3197-3207.
- Tietz, N. W., Burtis, C. A., Ashwood, E. R. & Bruns, D. E. (2008). *Tietz: Fundamentos de Química Clínica*. 6a ed. Rio de Janeiro: Elsevier.
- Van Dyk, E., Bosman, A. M., Van Wilpe, E., Williams, J. H., Bengis, R. G., Van Heerden, J. & Venter, E. H. (2011). Detection and characterisation of papillomavirus in skin lesions of giraffe and sable antelope in South Africa. *Journal of the South African Veterinary Association*, 82(2): 80-85.
- Vickers, A. (2002). Botanical medicines for the treatment of cancer: rationale, overview of current data, and methodological considerations for phase I and II trials. *Cancer investigation*, 20(7-8): 1069-1079.
- Williams, J. H., Van Dyk, E., Nel, P. J., Lane, E., Van Wilpe, E., Bengis, R. G., De Klerk-Lorist, L. M. & Van Heerden, J. (2011). Pathology and immunohistochemistry of papillomavirus-associated cutaneous lesions in Cape mountain zebra, giraffe, sable antelope and African buffalo in South Africa. *Journal of the South African Veterinary Association*, 82(3): 97-106.
- Yacout, G. A., Elguindy, N. M. & El Azab, E. F. (2012). Hepatoprotective effect of basil (*Ocimum basilicum* L.) on CCl₄-induced liver fibrosis in rats. *African Journal of Biotechnology*, 11(90): 15702-15711.
- Yang, K., Lamprecht, S. A., Liu, Y., Shinozaki, H., Fan, K., Leung, D., Newmark, H., Steele, V. E., Kelloff, G. J. & Lipkin, M. (2000). Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. *Carcinogenesis*, 21(9): 1655-1660.