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Establishing the Human Normal Standard Laboratory Tests' Values following Orally Administered Protocatechuic Acid (PCA) Supplementation

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Abstract

Protocatechuic acid (PCA), is a natural phytochemical found throughout nature: in soil, plants, vegetables, streams, ponds and lakes. PCA is common to the human diet and is also produced in small amounts by bacteria in the human large intestine. PCA is non-toxic, non- allergenic and non-mutagenic and the side effects are minimal. It is generally recognized as safe by the FDA as a food flavoring additive (G.R.A.S.).

Keywords: Protocatechuic Acid (PCA); Soil; Plants; Vegetables; Food; Health; Wellness

Introduction

Protocatechuic acid (PCA), a food supplement was first reported in the literature in 1890 as referenced by Agmon et al. [1]. There are now over 1000 publications in the literature primarily based upon in vitro and animal studies concerning health and wellness [2,3].

Protocatechuic acid (PCA), is a natural phytochemical found throughout nature: in soil, plants, vegetables, streams, ponds and lakes [2,3]. PCA is common to the human diet [2,3] and is also produced in small amounts by bacteria in the human large intestine [4,5]. PCA is non-toxic, non- allergenic and non-mutagenic and the side effects are minimal. It is generally recognized as safe by the FDA as a food flavoring additive (G.R.A.S.) [6].

There have been many health benefits reported on the use of PCA, including having a wide range of pharmacological activities such as being an antioxidant [2,3,7,8] and anti-inflammatory properties [9,10]. PCA has been reported to have the following properties; anti-arthritic [3], analgesic [10], neuroprotective, antibacterial, antiviral, anticancer, anti-osteoporotic, having antiaging activities, protection from metabolic syndrome, and preservation of liver, kidneys, and reproductive functions [2,3].

The PCA-rich phenolic compounds extracted from different natural herbs have showed immune- enhancing and anti-inflammatory properties that can be used as natural feed additives [11,12].

The mechanism for these antioxidant properties may be due to the increased phagocytosis in the presence of PCA or by the secretion of immunoglobulin and inhibition of the pro-inflammatory cytokine secretion [13].

PCA is a powerful antioxidant. Antioxidants are fundamental to health and wellness. PCA is a powerful anti-inflammatory reagent. Inflammation is now known to be basis of all disease.

PCA has been introduced commercially for health and wellness. PCA is being studied for clinical applications. However, there are no human

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laboratory tests biomarkers in the literature critical for patient care. The available information from the literature is based primarily upon in vitro and animal studies. The reports indicated that PCA produces some abnormal standard laboratory tests biomarkers important for patient care. Decreased blood clotting [14,15], decreased blood glucose [16,17] and lowering of blood cholesterol [18] were reported. These reports if confirmed in humans would have significant bearing on clinical judgment and implementation. These and other standard laboratory biomarkers related to oral PCA administration need to be established in humans. They are relevant to patient care, especially in monitoring during surgical intervention.

Immunity is fundamental to human health and wellness. There may be a link between PCA's antiinflammation properties and immunity in humans [19] PCA's therapeutic effect on avian influenza was reported due to alterations in hormonal and cellular immunity in poultry [20,21]. PCA's potential effect upon human immunity needs to be explored.

The sandard laboratory biomarkers need to be validated in humans following treatment with PCA. Knowing the biological effects on the human structure and function are essential in human research and patient care. However, to our knowledge there is only one human PCA study in the literature. That was related to the reduction of Cutibacterium acnes bioburden on human skin by topical application of a PCA formulation but did not include standard laboratory biomarkers [22].

PCA has become commercially available as a food supplement for health and wellness purposes as well clinical studies. PCA is now produced biochemically. Therefore, establishing the standard laboratory biomarkers in humans becomes important. The purpose of this proof of principle pilot clinical study is to evaluate safety following two weeks of PCA oral supplementation. In addition, it is important to establish the metrics of routine laboratory biomarkers critical in clinical practice, especially those reported in the literature to be different from normal following PCA treatment. The study was approved by the Independent Ethics committee Dhanashree Hospital Pune, India.

Materials and Methods

A randomized controlled, double blinded proof of principle pilot study was initiated on a cohort of normal healthy men and women ages 50-60 following two weeks of oral PCA supplementation. Subjects were willing to continue with their existing diet, exercise and lifestyle habits throughout the study without any change.

The exclusion criteria were as follows.

1. Subjects with allergy or sensitivity to any ingredient of investigational product.
2. Participation in any clinical study within 30 days before randomization.
3. Pregnant and lactating women.
4. Any kind of substance abuse, (alcohol, tobacco, or any other substance).
5. Subjects with uncontrolled metabolic diseases or chronic diseases at the discretion of the investigator.
6. Subjects with unstable medical conditions at the discretion of the investigator.
7. Subjects with planned surgery during the trial or history of surgery in past 3 months.
8. Current diagnosis of immune compromised conditions; HIV, AIDS, or cancer.
9. History of blood/bleeding disorders; blood donation in previous 2 months.
10. Individuals who have had COVID-19 past three months.
11. Individuals who have a history of major arthritis or joint problem.
12. Any other condition that in the qualified investigator's opinion may adversely affect the participants ability to complete the study or its measures or which may have posed a significant risk to the participant.
13. All enrolled subjects will be instructed to take 4 capsules of 500 mg (2000mg) of PCA and Placebo, 2 daily in the morning and 2 in evening, 2 hours before breakfast and dinner for 14 days.

The clinical status and laboratory test biomarkers were taken before and after two weeks of oral administration of PCA. Biomarker laboratory results were obtained in both groups prior to any treatment to establish baselines. Then one arm received placebo tablets (N=14), and the other arm received PCA (1000 mg of oral PCA daily, N=12) for a period of two weeks. Following the two-week treatment, the PCA capsule group results are compared to the Placebo group. The dosage, interval and duration were consistent with the potential clinical application of perioperative nutritional optimization.

Monitoring and documentation of number and type of adverse events including changes in laboratory parameter (Complete blood count with differential, ESR, kidney function, liver function, lipid profile) from Baseline (Day 1) to the end (Day 14) of the study. In addition an evaluation was made as to the efficacy of PCA capsules as compared to placebo based on personal health assessment test through SF 36 Survey, to evaluate the efficacy of PCA capsules as compared to placebo based on laboratory examinations (HbA1c, fasting blood glucose & post prandial sugar, Hs CRP, IGF-1, IgA,

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IgG, CXCL9, GLP1 & Prothrombin time). Eligible subjects were assigned in 1:1 ratio to treatment with the test product, PCA Capsule or Placebo in this double- blind, randomized, placebo- controlled, parallel study. Subjects were required to use diaries to document the date, time and dosage of study treatments including any missed doses and the occurrence of any adverse events. The efficacy of investigational product was assessed primarily with HbA1c, fasting blood glucose & post prandial sugar, Hs CRP, IGF-1, IgA, IgG, CXCL9, GLP1 & Prothrombin time. Individual participant data was not shared publicly.

The results are reported from two groups, the PCA capsule group and the Placebo group. The first results were the base line laboratory test results for each group. The second results are those following two weeks of treatment, the PCA capsule group or the Placebo group. The entire detailed results are in the appendix.

Results

Compliance was good. At day 14 following treatment 96.2% of total study cases had 80-100% of compliance. There was 100.0% compliance in placebo group which was more than the 91.7% of cases in PCA Capsule group. At the end of the study, 100.0% of the cases in both groups had normal physical examinations.

PCA supplementation was safe. The FS-36 scores were unchanged by PCA treatment. Vital signs were unchanged by the PCA treatment. The blood tests, red blood cells, white blood cells and cellular morphology were unchanged by PCA treatment.

The following human laboratory test biomarkers were selected to be highlighted because they were different from that reported the literature and or are germane to future clinical application, prothrombin time, blood glucose, IGF-1, and blood cholesterol. Those therapeutic biomarkers that are routinely used during the standard of care are highlighted, i.e., erythrocyte sedimentation rate, HsC-reactive protein, and immunity biomarkers. Those laboratory test biomarkers that may have relevance in clinical care are highlighted, i.e., liver disease, globulins, systolic blood pressure, glucagon-like peptide-1 and weight loss.

Prothrombin time: This data suggests that at baseline, mean Prothrombin time was 11.97 sec among PCA Capsule which was comparable to 12.20 sec among Placebo group and the difference was not significant. After 14 days of treatment, mean Prothrombin time showed an insignificant rise of 4.4% among PCA Capsule and 3.3% in Placebo group from baseline. The change was comparable between the groups, and the difference was not significant.

Fasting Blood Glucose: In this analysis at baseline, mean Fasting Blood Glucose was 114.08 mg/dL among PCA Capsule which was comparable to 110.14 mg/dL among Placebo group and the difference was not significant. After 14 days of treatment, mean Fasting Blood Glucose showed an insignificant fall of 5.5% among PCA Capsule and 8.4% in Placebo group from baseline. If compared, the change was comparable between the groups, and the difference was not significant.

Post Prandial Blood Glucose: This profile states that at baseline, mean Post Prandial Glucose was 135.42 mg/dL among PCA Capsule which was insignificantly less as compared to 141.14 mg/dL among Placebo group. After 14 days of treatment, mean Post Prandial Glucose showed an insignificant rise of 0.5% among PCA Capsule and an insignificant fall of 4.4% in Placebo group from baseline. If compared, the change was insignificantly more among PCA Capsule than Placebo group.

Insulin-like Growth Factor 1 (IGF-1): This result states that at baseline, mean IGF-1 was 126.26 ng/mL among PCA Capsule group which was more as compared to 117.02 ng/mL among Placebo group, but the difference was not significant. After 14 days of treatment, mean IGF-1 showed an insignificant fall of 1.0% among PCA Capsule group and an insignificant rise of 1.8% in Placebo group from baseline. If compared, the change was insignificantly less among PCA Capsule than Placebo group.

Cholesterol: The cholesterol results were the only ones departing from normal that may have an adverse clinical effect. The elevation is contrary to the literature which reported PCA reduced the cholesterol. For these reasons the following extensive results on cholesterol are listed.

Total Serum Cholesterol OTAL (176.7): This profile states that at baseline, mean Total Serum Cholesterol was 194.75 mg/dL among PCA Capsule group which was insignificantly more as compared to 173.21 mg/dL among Placebo group. After 14 days of treatment, mean Total Serum Cholesterol showed a significant rise of 9.3% among PCA Capsule group and an insignificant fall of 3.8% in Placebo group from baseline. If compared, the change was significantly more among PCA Capsule group than Placebo group.

HDL Cholesterol (43.2): In this study at baseline, mean HDL Cholesterol was 41.63 mg/dL among PCA Capsule group which was comparable to 43.86 mg/dL among Placebo group and the difference was not significant. After 14 days of treatment, mean HDL Cholesterol showed an insignificant fall of 1.8% among PCA Capsule group and 10.1% in Placebo group from baseline. If compared, the change was insignificantly less among PCA Capsule than Placebo group.

NON HDL Cholesterol (133.5): In this study at baseline, mean Non-HDL Cholesterol was 153.12 mg/dL among PCA

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Capsule group which was more as compared to 129.36 mg/dL among Placebo group, but the difference was not significant. After 14 days of treatment, mean Non-HDL Cholesterol showed a significant rise of 12.3% among PCA Capsule group and an insignificant fall of 1.6% in Placebo group from baseline. If compared, the change was significantly more among PCA Capsule than Placebo group.

LDLL Cholesterol (110.5): In this study at baseline, mean LDL Cholesterol was 120.72 mg/dL among PCA Capsule group which was insignificantly more as compared to 100.03 mg/dL among Placebo group. After 14 days of treatment, mean LDL Cholesterol showed a significant rise of 14.7% among PCA Capsule group and an insignificant fall of 0.8% in Placebo group from baseline. If compared, the change was significantly less among Placebo group than PCA Capsule group.

Very-low-density lipoprotein (VLDL Cholesterol): In this study at baseline the mean VLDL Cholesterol was 32.40 mg/dL among PCA Capsule group which was comparable to 29.33 mg/dL among Placebo group and the difference was not significant. After 14 days of treatment, mean VLDL Cholesterol showed an insignificant rise of 3.5% among PCA Capsule group and an insignificant fall of 4.2% in Placebo group from baseline. If compared, the change was insignificantly more among PCA Capsule than Placebo group.

Erythrocyte Sedimentation Rate (ESR): This data suggests that at baseline, mean ESR was 10.83 mm/hr among PCA Capsule group which was comparable to 13.50 mm/hr among Placebo group and the difference was not statistically significant. After 14 days of treatment, mean ESR showed an insignificant rise of 68.5% among PCA Capsule group and a significant rise of 1.3 times in Placebo group from baseline. If compared, the change was more among Placebo group than PCA Capsule group, but the difference was not significant.

High Sensitivity C-Reactive Protein (Hs CRP): In this analysis at baseline, mean high-sensitivity C- reactive protein (Hs CRP) was 4.90 mg/L among PCA Capsule group which was comparable to 8.26 mg/L among Placebo group and the difference was not statistically significant. After 14 days of treatment, mean Hs CRP showed an insignificant fall of 54.3% among PCA Capsule group and 37.7% in Placebo group from baseline. If compared, the change was more among PCA Capsule group than Placebo group, but the difference was not significant.

Immunity Biomarkers: There was a 25% rise above normal for CXCL9 but not considered significant because of low numbers. There was no significant effect on Immunoglobin G (IgG) and Immunoglobin A (IgA).

CXCL9: (Normal at this laboratory is 0 to 700 pg/ml). As per this analysis at baseline, mean CXCL9 was 640.87 pg/mL among PCA Capsule group which was insignificantly more as compared to 545.04 pg/mL among Placebo group. After 14 days of treatment, mean CXCL9 showed an insignificant rise of 25.6% among PCA Capsule group for a total of 804.93. and 9.0% in Placebo group from baseline or total of 594.04. If compared, the change was more among PCA Capsule group than Placebo group, but the difference was not significant.

Alanine Aminotransferase (SGPT; 7-56): This result reveals that at baseline, mean SGPT was 19.84 U/Lt among PCA Capsule group which was comparable to 16.76 U/Lt among Placebo group and the difference was not statistically significant. After 14 days of treatment, mean SGPT showed an insignificant rise of 40.0% among PCA Capsule and an insignificant fall of 10.3% in Placebo group from baseline. If compared, the change was significantly more among PCA Capsule group than Placebo group.

Alkaline Phosphate: This analysis states that at baseline, mean Alkaline Phosphatase was 95.92 U/Lt among PCA Capsule group which was insignificantly more as compared to 82.57 U/Lt among Placebo group. After 14 days of treatment, mean Alkaline Phosphatase showed an insignificant rise of 4.1% among PCA Capsule group and an insignificant fall of 6.3% in Placebo group from baseline. If compared, the change was significantly more among PCA Capsule than Placebo group. The rise produced by PCA in normal volunteers will be important factor to consider in clinical practice.

Globulin: In this study at baseline, mean Globulin was 2.88 g/dL among PCA Capsule group which was comparable to 3.01 g/dL among Placebo group and the difference was not significant. After 14 days of treatment, the mean Globulin showed a significant rise of 6.6% among PCA Capsule group and an insignificant rise of 0.7% in Placebo group from baseline. This information should be a consideration.

Systolic blood pressure (SBP): This table states that at baseline, mean SBP was 135.17 mmHg among PCA Capsule group which was comparable to 136.00 mmHg among Placebo group and the difference was not significant. After 14 days of treatment, mean SBP showed a significant fall of 8.8% among PCA Capsule group and an insignificant fall of 4.8% in Placebo group from baseline. If compared, the change was comparable between the groups, and the difference was not significant.

Glucagon-like peptide-1 (GLP-1): According to this study at baseline, mean GLP-1 was 0.02 pmol/mL which was same among both the group and the difference was not significant. After 14 days of treatment, mean GLP-1 showed an insignificant fall of 4.2% among PCA Capsule group and insignificant rise of 9.1% in Placebo group from baseline. If compared, the change was less among PCA Capsule than Placebo group, but the difference was not significant. GLP-1 plays a role in glucose blood levels, appetite and weight loss [23].

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Discussion

PCA oral supplementation was safe. The side effects to all participants in this study was 11.5%. 25.0% of the PCA capsule group reported a side effect which was more than the Placebo group, but the difference was not significant. Severity of the side effects was mild to moderate among PCA capsule group. They were resolved during the treatment.

The standard laboratory test biomarkers in humans were established following two weeks of PCA food supplementation. Abnormal test results from the literature based upon in vitro and animal studies were not validated. Importantly the results in common laboratory test biomarkers in this human cohort were in the normal range. The human results differed from those in the literature of PCA's effect upon blood clotting, blood glucose, serum IGF-1, and blood cholesterol levels. The prothrombin time and blood glucose metrics were in normal range which are important during patient care. The cholesterol was slightly elevated rather than lowered. Contrary to animal studies there was no increase in the human serum IGF-1level that could have general anabolic effects, yet unknown or identified.

Of potential concern for future human clinical application was the minimal elevation of the blood cholesterol even though significant variations can exist based on age, region, and individual health factors. This elevation was contrasted with the previously reported decrease in blood cholesterol reported in the literature.18 It would be prudent to follow the cholesterol levels in patients being treated with PCA.

The literature report of PCA influence on GLP-1 levels was not confirmed in this human study. It should be noted that GLP-1 levels can fluctuate significantly throughout the day. Therefore, a single measurement may not be a reliable indicator of overall GLP-1 status. There are publications reporting the control of blood glucose and potential treatment for insulin resistant conditions and type 2 diabetes.16 However, this proof of principle pilot study did not support this contention. PCA's role in glucose control and weight loss is yet to be established in humans [22].

There is published evidence that PCA has benefit to the immune system in the poultry literature on avian flu [20,21]. CXCL9 an immunity biomarker was elevated numerically in this study suggesting PCA has an immunological property. CXCL9 is a monokine or chemokine ligand 9 has been examined as an alternative marker of immunogenicity [24]. The CXCL9 biomarker is used in assessing an immunological response to cancer therapies [25]. However, the immunological effect of in this study was not conclusive because of the small numbers and the other two biomarkers for immunity assessment, IgG and IgA were not altered.

The strength of this study was correcting the laboratory test biomarkers reported in the literature based only on animal and in vitro studies, not yet known in humans. This human evidence is important when clinical application of PCA is considered.

The weakness of this study is the small numbers inherent in a proof of principle pilot study. There were trends as with immunity, but it is not possible to apply statistical significance to small numerical values. Future studies with larger number would satisfy this weakness.

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Conflicts of Interest

This study was entirely funded by Pcabioscience, LLC of Las Vegas, NV to stablish safety and correct the literature necessary prior to clinical application. Dr. Johnson is the owner. Dr. Johnson holds multiple US patents related to protocatechuic acid. The study was performed on an independent contract basis with Pcabioscience's payment to ProRelix Services, LLP. Drs. Avhad and Thasma were paid by ProRelix as independent contractors functioning under work for hire status. Drs. Avhad and Thasma designed and executed this study without any financial interests. Drs. Avhad and Thasma have no conflict of interests.

Appendix File Link: **Click Here**

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